# AD-A240 299

AD A

CONTRACT NO:

DAMD17-89-C-9119

TITLE:

"SAFETY TESTING OF SEED AND VACCINES FOR DENGUE VIRUSES IN MICE, GUINEA PIGS, RABBITS AND BACTERIAL AND MYCOPLASMA CULTURE MEDIA"

PRINCIPAL INVESTIGATOR:

Louis Potash, Ph.D.

PI ADDRESS:

Program Resources, Inc. 7655 Old Springhouse Rd. McLean, Virginia 22102



REPORT DATE:

December 20, 1990

TYPE OF REPORT:

Final Report

PREPARED FOR:

U.S. Army Medical Research and Development

Command

Fort Detrick,

Frederick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

Approved for public release; distribution

unlimited

The view, opinion and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other documentation.

91 8 22 087

91-08770

REPORT D	OCTATION	I PAGE			Form Approved OMB No 0704-0188 Exp Date Jun 30 1986
1a. REPORT SECURITY CLASSIFICATION Unclassified		16. RESTRICTIVE A	MARKINGS	and the second second second	
2a. SECURITY CLASSIFICATION AUTHORITY		3. DISTRIBUTION	AVAILABILITY OF	REPORT	
2b. DECLASSIFICATION/DOWNGRADING SCHEDU	.E		for public i ion unlimite		e;
4 PERFORMING ORGANIZATION REPORT NUMBER	R(S)	5. MONITORING C	ORGANIZATION RE	EPORT NU	IMBER(S)
6a NAME OF PERFORMING ORGANIZATION Flow Laboratories, Inc. Program Resources, Inc.	6b. OFFICE SYMBOL, (If applicable)	7a. NAME OF MO	NITORING ORGA	NIZATION	
6c. ADDRESS (City, State, and ZIP Code) 7655 01d Springhouse Rd. McLean, VA 22102	6 0127	7b. ADDRESS (City	y, State, and ZIP (	Code)	
8a NAME OF FUNDING/SPONSORING ORGANIZATION US Army Medical	8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT		ENTIFICAT	TON NUMBER
Research & Development Command 8c. ADDRESS (City, State, and ZIP Code)	SGRD-RMI-S	DAMD17-89			
Fort Detrick Frederick, MD 21702-5012		PROGRAM ELEMENT NO. 63002A	PROJECT NO. 3M2 63002D810	TASK NO A	WORK UNIT ACCESSION NO C WUDA335465
16. SUPPLEMENTARY NOTATION  17 COSATI CODES  FIELD GROUP SUB-GROUP  06 03  06 13  19. ABSTRACT (Continue on reverse if necessary Preclinical safety testing o  1) 5 different cell culture guinea pigs; and 3) bacteria tests were the crude, unclari	18. SUBJECT TERMS (C Safety Testing: Dengue-1 and identify by block of f dengue virus p lines; 2) rabbit l fungal and myc fied harvests of	4. DATE OF REPO 90 Dec continue on reverse Hepatitis and Dengue broduction s ts, mice (ad coplasma cul f both contr	RT (Year, Month, 20  e if necessary and A; Vaccine; -4 Producti eeds consistult & newboture media. rol and viru	Day) 15 d identify Viral on See sted of orn suc Inoc is flui	by block number) Seeds; RAI; ds inoculation in: cklings) and cula for these ids. For dengue-1,
3 different PDK passage leve carried out following guidel vaccines as found in 21 CFR, A dengue-l vaccine lot was s kidney cell cultures in attetitered immune serum resultecells) for both viruses and	ines established Part 600 and we ubjected to 20 a mpts at further ed in unsatisfac	d by the FDA ere performe additional s attenuation tory finding	A for live and in accordance in accordance in tissue dengue-4 v	and ina dance wages (to with swith coulturings	activated virus with GLP regulatio total 30) in dog lack of high- ure purity (AGMK
20. DISTRIBUTION / AVAILABILITY OF ABSTRACT  UNCLASSIFIED/UNLIMITED SAME AS	RPT DTIC USERS		Unclassi	ified	
22a, NAME OF RESPONSIBLE INDIVIDUAL Virginia M. Miller	20 adition may be used up	226 TELEPHONE ( (301) 663			OFFICE SYMBOL GRD - RMI-S

# TABLE OF CONTENTS

I.	INTRODUCTION	2
II.	PRECLINICAL SAFETY TESTING	3
	DENGUE-1 VIRUS	3
	DENGUE-4 VIRUS	4
ıı.	SERIAL PASSAGES	4
IV.	CONCLUSIONS	5
ATTACHM	ENT A	6
ATTACHM	ENT B: Quality Assurance Statement from Program Resources, Inc	7
APPENDI	X I: Dengue-1 Virus Strain #45AZ5 pages 1 -	138
APPENDI	X II: Dengue-4 Virus Strain Carib 341750 pages 1 -	144

			1		
Accesion	n For				
NTIS		U		_	
f. ( )	<i>.1</i> -3	( )	1		
Uare	e ood	4	1	1200	(5)
J rud.				OOFY COTED	"]
				C.	<i>i</i>
Ву		## . * * * * * * * *			
Di t ib.	dio 1		1		
	*****				
A	zalta sudy. C	30° GE			
	A. S 3	er am man Lett	.——- 		
Diul	در ران				
1A-1					
7 7					
L	<u> </u>			ļ	

## I. INTRODUCTION

The Virus Vaccine Production Laboratory of Flow Laboratories, Inc. had been awarded a contract by USAMRDC to conduct preclinical testing services for Walter Reed Army Institute of Research (WRAIR) to evaluate dengue vaccines and seeds for purity, safety and potency in accordance with the technical proposal entitled "Safety Testing of Seed and Vaccines for Dengue Viruses in Mice, Guinea Pigs, Rabbits and Bacterial and Mycoplasma Culture Media". Effective Jan 1, 1990, this research, production and testing Laboratory was purchased by Program Resources, Inc. (PRI) and this contractual effort continued under an approved subcontract with PRI.

が教育的 とび HERMAN から 大変な というな

\_)

The preclinical testing services consisted of the inoculation of submitted test articles in: 1) five different cell culture lines; 2) mice (adult and new-born sucklings), guinea pigs and rabbits; and 3) bacterial, fungal and mycoplasma culture media. The inocula for these tests were crude, unclarified harvests of both control and virus fluids plus specific immune serum. All tests were carried out following guidelines established by the FDA for live and inactivated virus vaccines as found in 21 CFR, Parts 510.11, 610.12, 610.30, 630.10 - 630.18, 630.30 - 630.37, 630.40, 630.50 - 630.57 and 630.60 - 630.67 and were performed in accordance with Good Lacoratory Practices (GLP) regulations for preclinical testing of biologicals (21 CFR, Part 58).

Although initially designed as a three (3) year contract, this effort has been brought to a close within an 13 month period with the completion of the preclinical safety testing of the following fluids: production seed pools of 3 different PDK passage levels grown in FRhL-2 cells of both dengue-1 (#45AZ5) and dengue-4 (CARIB #341750) viruses.

In addition, in an effort to attenuate a dengue-1 vaccine lot which had previously undergone 10 serial passages in dog kidney (DK) cell cultures, the virus was scrially passaged 20 more times (total 30 passages) in DK cell cultures with aliquots of each passage level submitted to the CCR, as directed. It was 3 of these 30 DK passage level harvests that served as the inocula for the above dengue-1 production seeds in FRhL-2 cell cultures.

## II. PRECLINICAL SAFETY TESTING

Over the 13 month period, a total of 8 test articles, composed of 6 crude, unclarified virus fluids and 2 related, crude, unclarified control fluids, was safety tested. As specified in the contract workscope, these fluids were tested for:

a) microbial sterility (bacterial, fungal and mycoplasmal);

b) purity (safety) in tissue cultures (four tissue culture systems - AGNK, PHA, PRK and Flow 5000 plus the cell system in which the virus was grown - FRhL-2);

c) animal safety in rabbits, mice (adult and newborn sucklings) and guinea pigs.

The tests articles consisted of 3 dengue-1 virus production seeds plus a control fluid and 3 dengue-4 virus production seeds plus a control fluid. For the dengue-1 seeds grown in FRhL-2 cell cultures, the 10th, 20th and 27th dog kidney passage levels served as the specific inoculum. For the dengue-4 seeds grown in FRhL-2 cell cultures, the 6th, 10th and 15th dog kidney passage levels served as the specific inoculum.

## DENGUE-1 VIRUS (#45AZ5)

All fluids satisfactorily passed the microbial sterility tests. The results of the tissue culture purity (safety) tests were unsatisfactory only in the ACIK cell culture system with all 3 virus pools and were attributed to the failure of the supplied antiserum to completely neutralize the dengue-1 viruses. Eased on a previous test with a dengue-1 virus (strain Western Pacific 1974), difficulties with the AGMK purity test were anticipated; however, pre-treatment of the primary flask cultures with immune serum 24 nours prior to inoculation with neutralized virus did not prevent dengue virus-attributed cytopathology from occurring both in the primary flasks and in the secondary tube subcultures. As expected, the tube subcultures were completely resistant to challenge with the Coxsackie A-9 virus. All virus fluids satisfactorily passed the prescribed animal safety tests. Because the ACMK purity test was unsatisfactory, these fluids were not considered to have passed all the above prescribed preclinical tests. A Phase Report detailing all of the above test results is being submitted together with this Final Report.

## DENGUE-4 VIRUS (CARIB #341750)

Production Seed: PEK- 6, FRhL-2/d7 of 9 Mar 90

PDK-10, FRhL-2/d7 of 9 Mar 90

PDK-15, FRhL-2/a7 of 9 Mar 90

Control Fluid ..... of 9 Mar 90

All fluids satisfactorily passed the microbial sterility tests. The results of the tissue culture purity (safety) tests were unsatisfactory only in the AGMK cell culture system with all 3 virus pools and were attributed to the failure of the supplied antiserum to completely neutralize the dengue-4 viruses. Based on previous tissue culture purity tests with dengue viruses, difficulties with the AC!K purity test were anticipated. Dangue virus-attributed cytopathology was detected in the primary flasks us non-describt morphological changes and in the secondary tube subcultures as lytic changes. As expected, the cube subcultures were completely resistant to challence with the Coxsackie A-9 virus. All virus fluids satisfactorily passed the prascriced animal safety tasts in rabbits, adult mice and guinea pigs out results were inconclusive in suckling mice. The difficulties in the suckling mice tests, where many of the sucklings were found either dead, moriound of lethargic, were attriuted to the failure of the supplied antiserum to completely neutralize the dangue-4 viruses. Bacause the AGNK purity test was unsatisfactory and the suckling mice tests were considered inconclusive, these fluids were not considered to have passed all the above prescribed preclinical tests. A Phase Report detailing all of the above test results is being sucmitted together with this Final Report.

## III. SERIAL PASSAGES

Dengue Virus Type 1, Strain #45AZ5: Live-Attenuated Vaccine, Lot No. 1-82, Run 2. As an adjunct to these Preclinical Safety Tests, this laboratory continued the serial passages of this virus in dog kidney cell cultures in an effort to achieve further attenuation. Pre-screened, frozen ampules of primary dog kidney (PDK) cells (LOT 222) had been suplied by the COR. All studies were carried out in accordance with protocols submitted by the COR and included passaged control cultures. Commencing with the 10th serial passage harvest fluid produced during the previous contract, the laboratory successfully completed 20 additional serial passages using both 1st and 2nd passage DK cell cultures. Multiple 2 ml vials of each passage level (day 7 harvests of both virus infected and control cultures) were submitted to the CCR.

## IV. CONCLUSIONS

The praclinical safety testing of dengue virus production seeds in accordance with the specified workscope were completed. The test articles consisted of crude, unclarified harvest fluids of 3 different PDK passage levels of both lengue-1 and dengue-4 viruses grown in the FRhL-2 cell system. Due to the lack of high-titered, specific immune sera, unsatisfactory results were reported for the AGMK tissue culture purity (safety) tests on all 6 cangue virus fluids assayed. In addition, the inconclusive results obtained in the suckling mice test with the dengue-4 fluids - many of the sucklings were found either dead, moribund or lethargic - are attributed to this same lack of immune serum. It is imperative that, for any future preclinical safety testing of dengue virus fluids whether production seeds or vaccine fluids, high-titered, specific immune sera be made available so as to ensure the satisfactory completion of all the prescribed tests.



CORPORATE OFFICES
Life Sciences Center
9900 Blackwell Road • Rockville • Maryland 20850
(301) 738-1000 • Telex 908793 • Fax (301) 738-1036

BETHESDA LABORATORIES 5221 River Road • Bethesda • Maryland 20816 (301) 654-3400 • Telex 908793 Fax (301) 654-6916

December 17, 1990

Dr. Louis Potash Program Resources, Inc. Biomedical Services Division 7655 Old Springhouse Road McLean, VA 22101

Dear Dr. Potash,

Microbiological Associates, Inc. is an AAALAC accredited animal facility, and all studies are performed in accordance with the "Guide for the Care and Use of Laboratory Animals", U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, NIH Publication No. 86-23.

Sincerely,

Mary D. Whiteman

Study Director, In Vivo Assays

Biotechnology Division



# Program Resources, Inc.

Biomedical Services Division
7655 Old Springhouse Rd.
McLean, VA 22102 • (703) 506-0190
FAX (703) 506-0194

May 14, 1991

TO:

Mr. Donald Holzworth, Vice President

Dr. Louis Potash, Study Director

FROM:

James R. Plautz

Sr. QA Advisor

RE:

GLP Compliance Audit of Final Reports for Safety Testing

of Dengue Virus Type 1 and Type 4

Janush Claut May 14, 1991

On April 14, 1991 a complete audit for GLP compliance (21 CFR, Part 58) was conducted for the subject final reports and their respective raw data.

Our complete findings indicate that the studies were conducted under the guidance of the referenced Standard Operating Procedures (SOPs), the variations from the SOPs had no apparent effect on study outcome, and that the final report for each study is substantiated by the raw data.

Animal safety testing was conducted and reported separately from these final reports.

# APPENDIX I

Dengue-1 Virus Strain #45AZ5

## FORENCRD

Opinions, interpretations, conclusions and mocommendations are those of the author and are not necessarily endorsed by the U.S. Army.

Where coverighted material is quoted, permission has been obtained to use such material.

Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or serices of these organizations.

In conducting research using animals, the investigator(s) adhered to the "Guide for the Cars and Use of Laboratory Animals", prepared by the committee on the Cars and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Resource Council (NIM Publication No. 35-23, Reviseu 1985) - (see Attachment A).

For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45 CFR 46.

PI Signature

Date

# TABLE OF CONTENTS

	Forew	ord	•••••••••	1
I.	Intro	duci	tion	3
II.	Synop	sis	••••••	4
III.	Diffe	rent	Summary Relating to the Safety Testing of Three (3) t Passage Levels of Dengue Virus Type 1 (#45AZ5) on Seeds: Propagated in DBS-FRhL-2 Cell Cultures	6
	A.	Inc	ocula	6
	В.		fety Testing Procedures and Results on the Crude rvest Fluids	6
		1.	Microbial Sterility	6
		2.	Identity in Tissue Culture (Serum-neutralization)	7
		3.	Purity (Safety) in Tissue Cultures	7
		4.	Animal Safety	9
Table	s:Ia	nd :	II	10
Appen	dixes:	A		16
		В	••••••	20
		С	••••••	54
		D		94
Attac	hment:	A		137
Attac	chment:	В		138

## I. INTRODUCTION

The accompanying protocol is a description of the safety testing of 3 crude harvest lots of dengue virus type 1 designated as:

Dengue Virus Type 1 (#45AZ5): PDK-10, FRhL-2/d7: PDK-20, FRhL-2/d7 and PDK-27, FRhL-2/d7 of 16 Feb 1990

Utilizing the testing procedures herein described, this fluid is considered to have not passed satisfactorily all tests for safety including purity. The detailed records with respect to passage history, pool production, and subsequent safety testing may be found in the Paperatory notebooks located at:

The Walter Reed Army Institute of Research (WRAIR), Bldg. 501, Washington, DC 20307-5100 - (Dr. Ken Eckels)

The Experimental Virus Vaccine Production Laboratory - Suite #500 - (Flow Laboratories, Inc.) Program Resources, Inc. [PRI], McLean, VA - (Dr. Louis Potash)

All procedures performed at PRI followed Good Laboratory Practices (GLP) regulations (21 CFR, Part 58) and were carried out in accordance with the guidelines established by the FDA for live and inactivated vaccines as found in 21 CFR, Parts 610.11, 610.12, 610.30, 630.10 - 630.17, etc. of April 1989. These procedures are detailed in the following SOPs and recorded on the indicated VVPL Forms:

```
SCP No.: 500.001 - Issued 29 Oct 1980, Revised 13 Feb 1986
        500.002 - "
                        29 Oct 1980, "
                                            13 Feb 1986
        500.008 - "
                                      11
                        13 Jan 1981,
                                            3 Mar 1986
 VVPL FORM #008 - Issued 29 Cct 1980, Revised 3 May 1984
                        15 Jan 1981, " 13 July 1984
           016 -
                   ~11
                                      11
           017 -
                        16 Jan 1981,
                                           13 Jan 1986
           019 -
                        8 Oct 1984
```

## II. SYNOPSIS

A. Crude Virus Harvests:

Dengue Virus Type 1 (#45AZ5) PDK-10, FRhL-2/d7 of 16 Feb 90 PDK-20, FPhL-2/d7 of 16 Feb 90 PDK-27, FRhL-2/d7 of 16 Feb 90

- B. Safety Tests on Crude Harvest Fluids:
  - 1. Sterility: Fluid Thioglycollate (FTM), Tryctone Sova Broth (TSB), Myccolasma

No Growth
,10 02 011 011
No Growth
No Growth
No Growth

 Tissue Culture Identity and Purity (Safety): AGMK, PHA, FRhL-2, PRK, and Flow 5000.

a.	PDK-10 Virus Fluid	(25 ml)	Unsatisfactory*
υ <b>.</b>	PDK-20 Virus Fluid	(25 ml)	Unsatisfactory*
C.	PDK-27 Virus Fluid	(25 ml)	Unsatisfactory*
d.	Control Fluid (TCF)	-(25 ml)·	Satisfactory

- 3. Animal Safety:
  - a. Rabbits: I.D. & S.Q. (Appendix 8)

(1)	PDK-10	Virus	Fluid	(30 ml)	Satisfactory
(2)	PDK-20	Virus	Fluid	(30  ml)	Satisfactory
(3).	PDK-27	Virus	Fluid	(30 ml)	Satisfactory

b. Adult Mice: I.C. & I.P - (Appendix - C)

(1)	PDK-10 Virus Fluid	(10.6  ml)	Satisfactory
(2)	PDK-20 Virus Fluid	(10.5 ml)	Satisfactory
(3)	PDK-27 Virus Fluid	(10.6 ml)	Satisfactory

<sup>\*</sup> Test unsatisfactory only in the AGMK test system. Non-descript morphological changes observed in primary AGMK flask cultures, particularly after films were stained. All AGMK tube subcultures exhibited 2-3+ cytopathology.

Both flask and tube subcultures were negative for hemadsorption. All tube subcultures completely inhibited the Coxsackie A-9 challenge virus.

## 3. Animal Safety (continued):

```
c. Suckling Mice: I.C. & I.P. - (Appendix - C)
    (1) PDK-10 Virus Fluid*
                                 (2.2 \text{ ml})
                                                    Satisfactory
    (2) PDK-20 Virus Fluid*
                                 (2.2 \text{ ml}^2)
                                                    Satisfactory
    (3) PDK-27 Virus Fluid*
                                 (2.2 ml<sup>2</sup>)
                                                    Satisfactory
d. Guinea Pigs: I.C. & I.P. - (Appendix - D)
                                                    Satisfactory
    (1): PDK -6 Virus Fluid
                               (15.3 ml)
    (2) PDK-10 Virus Fluid
                                 (15.3 ml)
                                                    Satisfactory
    (3) PDK-15 Virus Fluid
                                 (15.3 \text{ ml})
                                                    Satisfactory
```

<sup>\*</sup> Virus fluid was mixed with equal parts of a 1:5 dilution of the immune serum and incubated at 37°C for 90 minutes prior to inoculation.

III. DETAILED SUMMARY RELATING TO THE SAFETY TESTING OF THREE (3)
DIFFERENT PASSAGE LEVELS OF DENGUE VIRUS TYPE 1 (#45AZ5)
PRODUCTION SEEDS: PROPAGATED IN DBS-FRhL-2 CELL CULTURES

## A. Inocula

In May 1990, the following frozen materials were obtained for testing from Dr. K. Eckels, Contracting Officer's Representative, Walter Reed Army Institute of Research (WRAIR), Bldg. 501, Washington, D.C.:

- Dengue-1 (#45AZ5) crude, unclarified harvest fluids of 16 Feb 1990:
  - a. PDK-10, FRhL2-2 (day 7 harvest)...... 20 x 10 ml vials
  - b. PDK-20, FRhL2-2 (day 7 harvest)...... 20 x 10 ml vials
  - c. PDK-27, FRhL2-2 (day 7 harvest)...... 20 x 10 ml vials
  - d. Control Fluids ...... 4 x 25 ml vials
- 2. Dengue-1 Antiserum: Jamaica HMAF of 4/17/73 ... 1 x 8 ml

On arrival in this laboratory, the virus and control fluids were stored at  $-70^{\circ}$ C, or below, and the antiserum at  $-20^{\circ}$ C, or below.

- B. Safety Testing Procedures and Results on the Crude, Unclarified
  Harvest Fluids (SOP No.: 500.008)
  - 1. Microbial Sterility (VVPL FORM #011)

Aliquots of the bulk frozen fluids were thawed and tested for microbial sterility as follows:

- a. Fluid Thioglycollate Medium FTM (LOT VVPL #030): Each of 10 culture tubes (9-10 ml medium per tube) was inoculated with 1 ml volumes of the crude virus fluids and each of 10 culture tubes was inoculated with 1 ml volumes of the crude control fluid. An additional 10 culture tubes were included as uninoculated controls. All cultures were vortex mixed and incubated at  $32^{\circ}$ C ( $\pm$   $2^{\circ}$ C) for 21 days with periodic examination for evidence of growth. No growth was observed in any of the 50 culture tubes.
- b. Tryptone Soya Broth TSB (LOT VVPL #030): Each of 10 culture tubes (9-10 ml medium per tube) was inoculated with 1 ml volumes of the crude virus fluids and each of 10 culture tubes was inoculated with 1 ml volumes of the crude control fluid. An additional 10 cultures were included as uninoculated controls. All cultures were vortex mixed and incubated at 22°C ( $\pm$ 2°C) for 21 days with periodic examination for evidence of growth. No growth was observed in any of the 50 culture tubes.

The results of the above described Microbial Sterility Assays are summarized in Table I.

c. Mycoplasma Sterility: These assays were performed by PRI's Mycoplasma Testing Laboratory and included both the routine PPLO agar and broth assays and the specific test for the detection of  $\underline{M}$ . Myorhinis. Samples (1 x 2 ml and 1 x 25 ml) of the 3 crude virus fluids and of the 1 control fluid were submitted for testing. All samples were reported to be negative for mycoplasmas. A copy of this report is appended to this Protocol - (Appendix A - 1, 2, 3 & 4).

## 2. Identity in Tissue Culture (Serum-Neutralization) -

No attempt was made to identity the crude virus pools in tissue cultures.

## 3. Purity (Safety) in Tissue Cultures - (VVPL FORM #016)

a. <u>Tissue Cultures</u>: All flask and roller tube cell cultures were prepared by contract personnel. Cultures were maintained on Medium MEM containing 5 to 10% fetal boving serum (heat-inactivated) plus antibiotics: gentamicin @ 100 mcg/ml; neomycin @ 50 mcg/ml; and amphotericin B (I.V.) @ 2.5 mcg/ml. Cultures were inoculated, roftd and subpassaged as indicated below. The following tissue culture systems were utilized:

(1)	Tertiary African Green Monkey Kidney (AGMK)	MEM	+	5%	serum
	Primary Human Amnion (PHA)				
(3)	Fetal Rhesus Lung (FRhL-2)	MEM	+	5%	serum
(4)	Primary Rabbic Kidney (PRK)	MEM	+	58	serum
(5)	Whole Human Embryo Fibroblast (Flow 5000)	MEM	+	58	serum

## b. General Testing Procedures

## (1) Crude Virus Fluids

(a) Primary Flask Cultures: Equal 5 ml volumes of the bulk crude virus fluids and of a 1:5 dilution of the rabbit immune serum (Den-1, Jamaica HMAF) were well mixed and incubated at 37°C (water eath) for 90 minutes. Due to the small volume of antiserum available, only 5 ml of each the virus fluids were tested per tissue culture system wherein 1 x 75 cm² flask per tissue culture system was inoculated with 10 ml of these serum-virus mixtures. These flasks were pre-treated 24 hours earlier with 0.5 ml of undiluted immune serum and overlayed with 25 ml of maintenance medium. Cultures were incubated at 35°C (37°C for PMA) for 14 days with periodic microscopic examination for any signs of CPE and/or collular degradation. When necessary to maintain the integrity of the cell films, cultures were refed with 35 ml of fresh medium.

(b) <u>Secondary Tube Subcultures</u>: On the 14th day of incubation, the primary cultures were re-examined microscopically and the fluids harvested individually and treated with the specific immune serum - 0.1 ml per harvest. In addition, to each individual harvest was added: 0.1 ml gentamicin (50 mg/ml); 1 ml penicillin-streptomycin solution (5000 units/ml and 5000 mcg/ml, respectively); and 10% of 10X SPG\* (v/v).

<sup>\* 10%</sup> SPG: sucrose, 2.13  $\rm H$ ;  $\rm KH_2PO_4$ , 0.038  $\rm H$ ;  $\rm K_2HPO_4$ , 0.072  $\rm M$ ; monoscalum glucamate, 0.049  $\rm M$ .

Following mixing, the fluids were incupated at coom temperature for 60 min, and then subpassed into homologous coller tube cultures - 0.5 ml of each harvest into each of 20 tubes. The cemainder of the harvest fluids was saved and stored at -75°C, or below. All primary cultures were tested for hemadsorption by the addition of 0.1% guinea pig RBC (in PBS) and incupation at 4°C for a minimum of 30 minutes. All cultures were negative for hemadsorption.

Tube cultures (refed with 2 ml of maintenance medium prior to inoculation) were incubated at 35°C (37°C for PHA) for 13-14 additional days. When necessary to maintain the integrity of the cell films, cultures were refed with 2 ml of fresh medium. Cultures were examined microscopically at periodic intervals and at the end of the incubation period for any signs of CPE. After final examination, tubes were divided - depending on the specific cell system - for additional testing:

ACMK, PHA, FRhL-2 and Flow 5000 Tube Cultures: These were divided into 3 groups as follows:

1/4th tested for the presence of hemadsorbing agents,

1/4th fixed and stained with a solution of 5% glutaraldehyde + 1:10 giemsa stain and examined microscopically for any CPE,

1/2 Challenged with Coxsackie A-9 virus (0.2 ml per tube at dilutions noted in the tables) for the detection of non-CPE producing agents and/or latent agents via the interference phenomenon.

PRK Tube Cultures: These were equally diviced into 2 groups:

1/2 tested for the presence of hemadsorbing agents,

1/2 fixed and stained with the glutaraldehyde-giemsa stain solution and examined microscopically for any CPE.

No challenge studies were carried out with the Coxsackie A-9 virus since this virus does not produce any discernible CPE in this tissue culture system.

## (2) Crude Control Fluid

A single 75 cm<sup>2</sup> flask per tissue culture system was incculated with 10 ml of crude control fluid. Cultures were handled in a manner similar to that described above for the crude virus fluid except that immune serum was not included.

## (3) Uninoculated Cell Lot Controls

Two x 75 cm<sup>2</sup> flasks per tissue culture system were included as uninoculated cell lot controls and were handled in a manner similar to that described above for the crude virus fluid except that immune serum was not included. In addition, an appropriate number of uninoculated roller tube cultures were included as cell lot controls for the secondary tube subcultures.

In all challenge studies, 1 to 4 culture tubes per set were left unchallenged to serve as controls to the challenge virus.

The results of these in vitro Tissue Culture Purity (Safety) tests are summarized in Tables II-A through -E.

## 4. Animal Safety Tests

Due to the dismantling of Flow's Animal Pacility during December 1989, all animal studies were performed by Microbiological Associates, Inc. The inocula for these animal studies were the three crude virus suspensions

- a. Adult Rabbits MSA Studies #ZA356.005101, #ZA357.005101 and #ZA358.005101 these tests were reported to be satisfactory and copies of these Final Reports may be found in Appendix 8.
- b. Adult and Suckling Mice MBA Studies #ZA356.005100, #ZA357.005100 and #ZA358.005100 all three tests in both adult mice and in suckling mice were reported to be satisfactory and copies of these Final Reports may be found in Appendix C.
- c. Adult Guinea Pigs MBA Studies \$ZA356.005102, \$ZA357.005102 and  $\frac{\$ZA358.005102}{$}$  these tests were reported to be satisfactory and copies of these Final Reports may be found in Appendix D.

Microbial Starility Test Results on the Crude Dengue-1 Virus (#45AZ5) Production Seed Pools Table I.

months of the control	i	Vol. per culture			Date	•
כנודרמו פ נופרוזמווו	22	(1817)	Temperature	Co Test	Orr Test	Results
Fluid Thicellycollate						
(FTR) LOT VVPL-#030	70	1	35°C (±2°C)	11/12/90	12/03/90	No Growth
PDK-10 Virus Fluid	10	1.0		_	_	No Growth
PDK-20 Virus fluid	3.0	1.0		_		No Growth
PDK-27 Virus Fluid	10	1.0		_		No Growth
Control Fluid	10	1.0		11/12/90	12/03/90	No Growth
						***************************************
Tryptone Soya Broth						
(TSB) LOT VVPL #030	10	\$44 Can	$22^{\circ}$ C ( $\pm 2^{\circ}$ C)	11/12/90	12/0	ar are wen
PDK-10 Virus Fluid	10	1.0				o Coowth
PDK-20 Virus Fluid	10	1.0				No Growth
PDK-27 Virus Fluid	10	1.0				No Growth
Control Fluid	70	1.0		11/12/90	12/03/90	No Growth

Tissue Culture Purity (Safety) Test Results on the Crude Dengue-1 Virus (#45AZ5) Production Seed Pools Table II.

A. Tertiary African Green Monkey Kioney (AGMK)

Complete inhibition of Coxsackic A-9 challenge virus by virus/serum mixture series. Coxsackie A-9 Challonge Results based on a 6-day incubation at 35°C. Prior to challenge, all tubes refed with 2 ml of fresh medium

Non-descript cytopatholgy initially observed on day 10 and confirmed on staining on day 14 for all 3 virus/serum inoculated flasks only. \*

cytopathology, confirmed on staining, was attributed to dengue virus breakthroughs. Islands of cells remained which proved to be resistant to the Commackie A-9 challenge virus. On day 20 (days 14 + 6), all tubes inoculated with harvests from viral/serum inoculated flasks exhibited cytopathology which progressed to 3-4+ by day 23 (days 14 + 14). This \*\*\*

Tissue Culture Furity (Safety) Test Results on the Crude Dengue-1 Virus (#45£,5) Production Seed Fools Table II.

B. Primary Human Amnion (PHA)

Coxsackie A-9 Challenge Results based on a 4-day incubation at 37°C. Prior to challenge, all tubes refed with 2 ml of fresh medium.

Tissue Culture Purity (Safety) Fest Results on the Crude Dengue-1 Virus (\$45A25) Froduction Seed Pools Table II.

C. Feral Rhesus Lung (FRhL-2)

The state of the s						0.5 ml per tude	ir tube			
	In	itial Fl.	asks			Passage #1	1,1			
•	Lot	\$ 1610 p21	Ţ	Lct # 1687	687 p24					
	Day 1	14		Dey 14	14 + 14 = 28	28				
							Coxea	ckie A-	Coxsackie A-9 Challenge*	*egne
Material Tested	CP	Hads	Stain	СРЕ	Hads	Stain	10-2	10-3	10 4	30-5
Court of Manager And Andrews		1/0	Ç	0/20	۶ / ۵	0/5	2/2	2/2	2/2	0/2
		1/0	9 €	61/0	) ) (	) ()	2/2	2/2	2/2	6/2
FIX-20 VICUS/Setuli SIXCUCS FIXE-27 Vicus/Setuli SIXCUCS		7.5	3 5	0/20	(S)	s >0	2/2	2/2	2/2	0/2
Control Fluid (TCF)	0/1	6/1	2	0/20	0/5	0/5	2/2	2/2	2/2	0/2
Control - (1)	2/0	6/5	37	0/40	0/10	0/10	4/4	4/4	4/4	3/4
Control - (2)		•		0/20	0/12	0/12	8/8	8/3	8/3	3/1

Coxsackie A-9 Challenge Results based on a 3-day incubation at 35°C. Prior to challenge, all tubes refed with 2 ml of fresh modium.

Tissus Culture Purity (Safety) Test Results on the Crude Dengue-1 Virus (#45AZ5) Production Seed Pools Table II.

D. Primary Rabbit Kichey (PRK)

1650	Inicial Flasks		0.5 n Pas	0.5 ml per tube Passage #1	e e
Material Tested CPE Harmon Day: 14  PDK-10 Virus/Serum Mixture 0/1 (PDK-27 Virus/Serum Mixture 0/1 (Control Fluid (TCF) (Control Fluid (TCF) (Control - (1) 0/2 (	Lot # 1650		Lot # 1693	593	
PDK-10 Virus/Serum Mixture 0/1 PDK-20 Virus/Serum Mixture 0/1 PDK-27 Virus/Serum Mixture 0/1 Control Fluid (1CF) (1	Day: 14		Day: I	2ay: 14 + 14 = 28	23
0/1 0/1 0/1 0/2	CPE Hads	Stain	CPE	Hacis	Stain
0/1 0/1 0/2	0/1	ON	0/20	07/0	0/10
0/1	0/1	Q.	0/20	0/10	0/10
0/1	1/0	Q.	0/20	0/10	0/10
1) 0/2		Q.	0/20	0/10	0/10
2)	0/2 0/2	GN	0/40	0/20 0/12	0/20

Tissue Culture Purity (Safety) Test Results on the Crude Dengue-1 Virus (#45AZ5) Production Seed Pools Table II.

E. Wiole Human Embryo Fibroblasts (Flew 5000)

0.5 ml per tube	Passage #1	# 1659 p20	12	Coxsackie A-9 Challenge*	Hads Stain $10^{-2}$ $10^{-3}$ $10^{-4}$ $10^{-5}$	5 0/5 2/2 3/2 3/2	5 0/5 2/2 2/2 2/2	5 0/5 2/2 2/2 2/2	0/5 0/5 2/2 2/2 2/2 1/2	0/10 0/10 4/4 4/4 1/4
				-9 Cha	10-4	2/2	2/3	2/2	2/2	4/4
				ckie A	- 1	3/2	2/2	2/2	2/3	4/4
cina z				Coxse	10-2	2/2	2/2	2/2	2/2	4/4
0.5 ml pe	Passago		23		;	0/5	o/ s	o/ 5	0⁄. 5	0/10
			+ 14 =		Hads	5 /0	0/5	0/5	o/ s	0/10
		Lot ? 1	• 6	i	CPE	0/20	0/20	0/30	0/20	0/40
	SKS	3			Stain	ND	ND ON	QI.	S	GIA
F	iciai Fi	1630 p.l	į		Hads	0/1	7	0/1	0/1	0/2
}	T	Q	Day 14		CPE	0/1	7/	0/1	1/0	0/2
	•		•	•	Material Tested	PDK-10 Virus/Serum Mixture	PDK-20 Virus/Serum Mixture	PDK-27 Virus/Serum Mixture	Control Fluid (ICF)	Control - (1)

Coxsackie A-9 Challenge Results based on a 5-day incubation at  $35^{\rm O}{\rm C}$ . Prior to challenge, all tubes refed with 2 ml of fresh medium.

PRI Program Resources, Inc.
Biomedical Services Division 7655 Old Springhouse Rd. McLean, VA 22102 • (703) 506-0190 FAX (703) 506-0194

8 August, 1990.

To: Dr. Louis Potash.

From: Jim Quartey. (50)

Subject: Mycoplasma Testing. (Charge # 807)

Agar testing were found to be negative.

This letter is to inform you that, the eight (8) samples listed below which you had submitted for the detection of Mycoplasma hyorhinis using the direct immunofluorescence staining and for the detection of Mycoplasma in general using the DNA Hoechst stain and

a. Dengue-1 (#45AZ5) Production Seed of 16 Feb 90:

- 1. PDK-10, FRhL-2/07.
- 2. PDK-20, FRhL-2/d7.
- 3. PDK-27, FRhL-2/d7.
- 4. Control Fluid.
- b. Dengue-4 (#341750) Production Seed of 9 Mar 93:
  - 1. PDK-6, FRhL-2/d7
  - 2. PDK-10, FRhL-2/d7
  - 3.PDK -15,FRhL-2/d7
  - 4. Control Fluid.

	Source No. 1868. Cates: 7/3/90 S	על קט פא	. Les	ic	يهي	الخ	7		,		9 <b>0</b>			: 5 Sept.	307
	Projected Final Rea	ding: Z	<u>/3/</u>	190	<u>&gt;</u>							-		E 807	•
	Billing Dates	}	311	Ling //E	Munic	er:			. ~	/3/				samples.	
		1.11.	Pr	112	inary				24.		Ļ		_	_	ļ
	Identification Number	11 -	Aqa	2	BTS			hst 2		2	day	Sub 1	2	Notes	
	Negative Control	1	0		0		0		0						-
	Positive Control		+-		+1		+		+	-		]			
NGUE	Positive Control (-/(#45AZ5) ,FRhL-2/47	197	0		0		0		0				}		
X-20	FRAL - 2/217	198	0		0		Q		0						
0K-2	7. FRAL-2/dJ	199	0		0		0		0						-
	DE FZUID	200	0		0		0		Ò						
•				-											
•															,
NGUE	-4 (#34/750) ,FRhL -2/d7	201	0		0		0		0	 	<b>-</b>		<del> </del>	<u> </u>	
	O, FRAL - 2/d7				2		0		0	ļ	<del> </del>				
	5, FRHL- 2/d7		Action of	/	0		0		0		<b>-</b>				
	ROL FLUID	204			0		D			<u> </u>		<del> </del>	<del>                                     </del>	<b> </b>	
<i></i>	RUL PLUID	207	U		$\mathcal{L}$		$\frac{\circ}{}$	ļ	0	ļ	-	<del>  -</del>	<del> </del>		
			-				-	ļ			╂—	ļ			
			-	-			<del> </del>		<b> </b>		├-	<del>                                     </del>	<del> </del>	<del> </del>	
					-		-	<u> </u>	<b> </b>		┼	-	-		
				-		. , ــــــ			<u> </u>	<u> </u>		-	<del> </del>		
	A Transfer of the Party of the						<del> </del>	}_	-	<u> </u>	<del> </del>	}_	<del> </del>	<b></b>	, <del></del>
		}					<b> </b> -	-		-	-	<u> </u>	-		
	Read by:	XXXXXXXX	~	<u>_</u> _		<u> </u>	K	}	K	<u> </u>	<del> </del>	-	1	<u> </u>	_
	Dates	XXXXXXXXX			8	-	157	<del>\</del>	\J\>	<u> </u>	-	-	<del> </del>	<b></b>	-77
		200000	<b>6</b> 7///	<u> </u>	125		/ss	1_	7/34		_		<u> </u>	1	<u> 131</u>
	NET: + = Postive  O = Negative  ? = Questional	, 91	4 <b>5</b> 0 '	Loc	No. 2									1486 Vin (	

Negative Control (-):/

Signed

# MYCOPLASMA TEST RECORD SHEET

National State of the State of

To the second

	No. ml Tested	Tested Date
Culture Medium	LOT # Aerobic	Aerobic   Anaerobic   On Test   Off Test   Results
DENGUE - 1(in	ギゲンススン)Virus Fluid	(#45AZS) Virus Fluid - LOT # PDK-10, FRAL - 2/27, MYC# 197
PPLO Agar	19005731 . 2	2 1 7 19 60 1 - 1 6.1 NEGATIVE.
PPLO Broth	1900503125.0	125.0 11/10 1/24/70 NEGATIVE
D 5 Subpass to Broth	0.501	125.0 1-1/1601-121/0 NEGATIVE
to Agar	2.	.2   //9/10   //3/70   NEGATIVE
D10 Subpass to Broth	125.0	125.0 1-1/26 0/2/0 NEGATIVE
to Fgar	27	. 2   //7/70 0/3/70 NEGATIVE
D15 Subpass to Broth	1.25.0	125.0 1 - 1911/6 01/6   NEGATIVE
to Agar	3.	1 . 2 1 1/27/20 NEGATIVE
DENGUE-1	H#5AZ5) Coule de Flui	2644865 Fluid - LOT # PUX-20 FRAL - 2/017. MYC#198
PPLO Agar	7.	NEGATIVE
PPLO Broth	1 25.0	125:0 1/1/40 1/24/90 NEGATIVE
D 5 Subpass to Broth	1 25.0	125.0 1 /11 6.1 -12.16.1 NEGATIVE
to Agar	3.	.2 1/16/70 1/31/70 NEGATIVE
D10 Subpass to Broth	1   25:0	1250 17/10/01/2/OCTIVE
to Agar	.2	1 . 2 1/17/70 19/3/70   NEGATIVE
D15 Subpass to Broth	1.25.0	125.01 -12116 18/8 6, UNEGATIVE
to Agar	2,	1 2 1 //24/10 9 9/10 I NEGATIVE
W.		FR 29101070
Positive Control (+)://o. cocgoneme	, engineme	Negative Control (-): / C O/ CO/O</td

# MYCOPLASMA TEST RECORD SHEET

No. ml Tested Date	
Culture Medium   LOT # .   Merobic   Anaerobic   On Test   Off Test   Res	Results
-/ (#45AZ5) virus Fluid - LOT # PDA	199.
1	ATIVE.
1900503 25.0  25.0  //01//-//N	NEGATIVE
s to Broth 1 125.0 125.0 1711/6/17/9/	NEGATIVE
10/10/10/10/10/10/10/10/10/10/10/10/10/1	NEGATIVE
h 1 125.0 125.0 17/9/6/18/9/0L	NEGATIVE
1 .2   .2   ////////	NEGATIVE
17/24618/8/60L	NEGATIVE
1.2 1/2/1/20/2/10	NEGATIVE
DENGUE - 1/#45AZS) control Fluid - LOT # MYC # 200.	
1 .2 1 .2 17/6 /2, 17/24/6,L	NEGATIVE
1 25.0 125.0 1/1/0 1/1/0	NEGATIVE
s to Broth   25.0   25.0   7/1, 6.1 7/2, 60	NEGATIVE
10/10/10/10/10/10/10/10/10/10/10/10/10/1	NEGATIVE
h 1 25.0125.0 17/9/b, 18/2/b,	NEGATIVE
10/1/10/1/10	NEGATIVE
h 1 25.0125.017/1/6,18/8/9/	NEGATIVE
1 ,21 ,21/2/7/101	NEGATIVE

FB 29101C070 Negative Control (-): Positive Control (+): M. O. O. Dinlin

# APPENDIX

В

## ANIMAL SAFETY TEST IN ADULT RABBITS

Study NO.: ZA356.005101						
Dengue-1 Prod Seed: PDK-10,	FRhL-2/d7	• • • • • • • • •	pages	21.	-	31
Study NO.: ZA357.005101 Dengue-l Prod Seed: PDK-20,	FRhL-2/d7	• • • • • • • •	pages	32	_	42
Study NO.: ZA358.005101						
Dengue-1 Prod Seed: PDK-27,	FRhL-2/d7		pages	43	_	53

## ANIMAL SAFETY TEST IN ADULT RABBITS

Study No.: ZA356.005101

Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7

Final Report For

Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

By Microbiological Associates, Inc. Life Sciences Center 9000 Blackwell Road Rockville, Maryland 20850

## TABLE OF CONTENTS

		Page
	Summary	23
I.	Introduction	24
II.	Study Information	24
III.	Procedures	25
IV.	Results	26
v.	Conclusions	27
VI.	Approvals	28
VII.	Quality Assurance Statement	31

## SUMMARY

The purpose of this assay is to detect the presence of adventitious agent(s) in the test article pre-clarified bulk live virus vaccine and/or fluids, other than the specific virus in the product. The test article was inoculated into adult rabbits.

No evidence of contamination with adventitious agent(s) was observed due to the test article Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7.

## I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent adventitious agent(s). The experimental design utilized inoculation of adult rabbits. The test is performed as described in CFR Title 21, Section 630.16.

Adult rabbits are utilized in this assay to detect possible contamination of the test article with B-virus or other adventitious agent(s) including other Simian agents, adenovirus(es), etc. which might be present in the test article. All animals are observed for signs of illness and any that become sick or show any abnormalities are examined in an attempt to establish cause of illness or death.

## II. STUDY INFORMATION

- A. Title: Animal Safety Test in Adult Rabbits
- B. Study Number: ZA356.005101
- C. Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7 was received at Microbiological Associates, Inc. 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.
- D. Medium Test Article: none
- E. Control Articles:
  - 1. Positive Control: none
  - 2. Negative Control: none
  - 3. Vehicle Control: none
- F. Test System: Rabbits, four females, SPF NZW, 1.5 -

2.5 kg.

Source: Buckshire Corp.

P.O. Box 155

Perkasie, PA 18944

G. Sponsor: Program Resources, Inc.

Biomedical Services Division

7655 Old Springhouse Road

McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department

Microbiological Associates, Inc.

Life Sciences Center 9900 Blackwell Road

Rockville, Maryland 20850

Animal Facility: Microbiological Associates, Inc.

5221 River Road

Bethesda, Maryland 20816

## I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate

Study Director: Janet Luczak, M.T. (ASCP),

M.G.A.

## J. Schedule:

Study Initiation Date: 07/16/90

2. Lab Initiation Date: 08/03/90

3. Lab Completion Date: 08/31/90

4. Study Completion Date: See Study Director's Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

## L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

## III. PROCEDURES

## A. Objective:

The study objective is to detect inapparent adventitious agent(s) which might be present in the test article, pre-clarified bulk live virus and/or fluids, other than the specific virus in the product.



## B. Methods:

1. Test System Identification and Randomization

Each rabbit was housed individually. Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Each rabbit was identified by a unique number tattooed on it's ear. The rabbit's number was recorded on the cage card.

The rabbits were randomized according to SOP #OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1.

Note: In error, animals were inoculated by intraocular route. This deviation from the protocol did not affect the outcome of this assay.

The rabbits were observed for 28 days for clinical signs of illness or distress.

## 3. Animal Husbandry

- a. Rabbits were fed certified rabbit chow <u>ad</u>
  <u>libitum</u> and water was supplied via water
  bottles, <u>ad libitum</u>.
- b. Rabbit's cages were changed weekly.
- c. Animal facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

## IV. RESULTS

All rabbits inoculated with the test article and the uninoculated control rabbit remained normal and healthy for the 28 day observation period.

See Tables 2 and 3 for a summary of the data.

#### ZA356.005101

#### V. CONCLUSIONS

No evidence of contamination with adventitious agent(s) due to the test article, Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7, was observed.

#### VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice Regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman Study Director

Date

# ZA356.005101

# TABLE 1

Group	No. of Animals	Routes of Inoculation	Volume of Inoculum	Inoculum	Treatment after Inoculation
1	1	I.D. S.Q. I.O.	1.0 ml Test 9.0 ml Artic 0.03 ml		Observe for Illness
2	1	SAA	SAA	SAA	SAA
3	1	SAA	SÃA	SAA	SAA
4	1	None	None	None	SAA

SAA = Same As Above

I.D. = Intradermal Inoculation S.Q. = Subcutaneous Inoculation

I.O. = Intraocular Inoculation

TABLE 2 Survival Summary for Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7

	RABBITS	
Test Article	3/3 <sup>a</sup>	
Uninoculated Control	1/1 <sup>b</sup>	

a Number of surviving, healthy animals after 28 days/Number of

animals inoculated.

b Number of surviving, healthy animals after 28 days/Number of animals on lab initiation date.

TABLE 3

Summary of Daily Observations
for Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post- inoc.)	Day of Death/ Sacrifice (Post- inoc.)
Rabbit	Test Article	1 2 3	Normal Normal Normal		
	Uninoc- ulated Control	4	Normal		

### QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT RABBITS

Study Number: ZA356.005101

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/19/90 - 07/19/90, TO STUDY DIR 07/19/90, TO MGMT 07/19/90 PHASES: PROTOCOL REVIEW

INSPECT ON 08/03/90 - 08/03/90, TO STUDY DIR 08/03/90, TO MGMT 08/06/90 PHASES: ADMINISTRATION OF TEST ARTICLE TO TEST SYSTEM

INSPECT ON 09/21/90 - 09/24/90, TO STUDY DIR 09/24/90, TO MGMT 09/28/90 PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Ed Warburton Date
Quality Assurance Unit

# ANIMAL SAFETY TEST IN ADULT RABBITS

Study No.: ZA357.005101

Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7

Final Report For

Program Resources, Inc. Biomedical Services Division 7655 Old Springhouse Road McLean, VA 22102

By Microbiological Associates, Inc. Life Sciences Center 9000 Blackwell Road Rockville, Maryland 20850

# TABLE OF CONTENTS

		Page
	Summary	34
I.	Introduction	35
II.	Study Information	35
III.	Procedures	36
IV.	Results	37
٧.	Conclusions	37
VI.	Approvals	38
VII.	Quality Assurance Statement	42

#### SUMMARY

The purpose of this assay is to detect the presence of adventitious agent(s) in the test article pre-clarified bulk live virus vaccine and/or fluids, other than the specific virus in the product. The test article was inoculated into adult rabbits.

No evidence of contamination with adventitious agent(s) was observed due to the test article Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7.



#### I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent adventitious agent(s). The experimental design utilized inoculation of adult rabbits. The test is performed as described in CFR Title 21, Section 630.16.

Adult rabbits are utilized in this assay to detect possible contamination of the test article with B-virus or other adventitious agent(s) including other Simian agents, adenovirus(es), etc. which might be present in the test article. All animals are observed for signs of illness and any that become sick or show any abnormalities are examined in an attempt to establish cause of illness or death.

#### II. STUDY INFORMATION

- A. Title: Animal Safety Test in Adult Rabbits
- B. Study Number: ZA357.005101
- C. Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7 was received at Microbiological Associates, Inc. 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.
- D. Medium Test Article: none
- E. Control Articles:
  - 1. Positive Control: none
  - 2. Negative Control: none
  - 3. Vehicle Control: none
- F. Test System: Rabbits, four females, SPF NZW, 1.5 -

2.5 kg.

Source: Buckshire Corp.

P.O. Box 155

Perkasie, PA 18944

G. Sponsor: Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road

McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department

Microbiological Associates, Inc.

Life Sciences Center 9900 Blackwell Road

Rockville, Maryland 20850

Animal Facility: Microbiological Associates, Inc.

5221 River Road

Bethesda, Maryland 20816

#### I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate

Study Director: Janet Luczak, M.T. (ASCP),

M.G.A.

#### J. Schedule:

1. Study Initiation Date: 07/16/90

2. Lab Initiation Date: 08/03/90

3. Lab Completion Date: 08/31/90

4. Study Completion Date: See Study Director's Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

# L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

#### III. PROCEDURES

# A. Objective:

The study objective is to detect inapparent adventitious agent(s) which might be present in the test article, pre-clarified bulk live virus and/or fluids, other than the specific virus in the product.



#### B. Methods:

1. Test System Identification and Randomization

Each rabbit was housed individually. Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Each rabbit was identified by a unique number tattooed on it's ear. The rabbit's number was recorded on the cage card.

The rabbits were randomized according to SOP #OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. The rabbits were observed for 28 days for clinical signs of illness or distress.

### 3. Animal Husbandry

- a. Rabbits were fed certified rabbit chow <u>ad</u>
  <u>libitum</u> and water was supplied via water
  bottles, <u>ad libitum</u>.
- b. Rabbit's cages were changed weekly.
- c. Animal facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

#### IV. RESULTS

All rabbits inoculated with the test article and the uninoculated control rabbit remained normal and healthy for the 28 day observation period.

See Tables 2 and 3 for a summary of the data.

#### V. CONCLUSIONS

No evidence of contamination with adventitious agent(s) due to the test article, Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7, was observed.

#### VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice regulations as found in Title 21 CFR Part 58.

Mary D./Whiteman

Study Director

ጥል	RT	.T.	1
10			

Group	No. of Animals	Routes of Inoculation	Volume of Inoculum	Inoculum	Treatment after Inoculation
1	1	I.D. S.Q.	1.0 ml 9.0 ml	Test Article	Observe for Illness
2	1	SAA	SAA	SAA	SAA
3	1	SAA	SAA	SAA	SAA
4	1	None	None	None	SAA

SAA = Same As Above

I.D. = Intradermal Inoculation

S.Q. = Subcutaneous Inoculation

TABLE 2

Survival Summary
for Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7

	RABBITS	
Test Article	3/3 <sup>a</sup>	
Uninoculated Control	1/1 <sup>b</sup>	

a Number of surviving, healthy animals after 28 days/Number of animals inoculated.

b Number of surviving, healthy animals after 28 days/Number of animals on lab initiation date.

TABLE 3

Summary of Daily Observations
for Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post- inoc.)	Day of Death/ Sacrifice (Post- inoc.)
Rabbit	Test Article	5 6	Normal Normal		
		7	Normal		
	Uninoc- ulated Control	4	Normal		

#### QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT RABBITS

Study Number: ZA357.005101

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/19/90 - 07/19/90, TO STUDY DIR 07/19/90, TO MGMT 07/19/90 PHASES: PROTOCOL REVIEW

INSPECT ON 08/03/90 - 08/03/90, TO STUDY DIR 08/03/90, TO MGMT 08/06/90 PHASES: ADMINISTRATION OF TEST ARTICLE TO TEST SYSTEM

INSPECT ON 09/21/90 - 09/24/90, TO STUDY DIR 09/24/90, TO MGMT 09/28/90 PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Ed Warburton 9-28-90

Ed Warburton Date
Quality Assurance Unit

# ANIMAL SAFETY TEST IN ADULT RABBITS

Study No.: ZA358.005101

Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7

Final Report For

Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9000 Blackwell Road
Rockville, Maryland 20850

# TABLE OF CONTENTS

		Page
	Summary	45
I.	Introduction	46
II.	Study Information	46
III.	Procedures	47
IV.	Results	48
v.	Conclusions	48
VI.	Approvals	49
VII.	Quality Assurance Statement	53

#### SUMMARY

The purpose of this assay is to detect the presence of adventitious agent(s) in the test article pre-clarified bulk live virus vaccine and/or fluids, other than the specific virus in the product. The test article was inoculated into adult rabbits.

No evidence of contamination with adventitious agent(s) was observed due to the test article Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7.

#### I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent adventitious agent(s). The experimental design utilized inoculation of adult rabbits. The test is performed as described in CFR Title 21, Section 630.16.

Adult rabbits are utilized in this assay to detect possible contamination of the test article with B-virus or other adventitious agent(s) including other Simian agents, adenovirus(es), etc. which might be present in the test article. All animals are observed for signs of illness and any that become sick or show any abnormalities are examined in an attempt to establish cause of illness or death.

#### II. STUDY INFORMATION

- A. Title: Animal Safety Test in Adult Rabbits
- B. Study Number: ZA358.005101
- C. Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7 was received at Microbiological Associates, Inc. 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.
- D. Medium Test Article: none
- E. Control Articles:
  - 1. Positive Control: none
  - 2. Negative Control: none
  - 3. Vehicle Control: none
- F. Test System: Rabbits, four females, SPF NZW, 1.5 -

2.5 kg.

Source: Buckshire Corp.

P.O. Box 155

Perkasie, PA 18944

G. Sponsor: Program Resources, Inc.

Biomedical Services Division

7655 Old Springhouse Road

McLean, VA 22102

Authorized Representative: Dr. Louis Potash



H. Testing Facility: Biotechnology Services Department

Microbiological Associates, Inc.

Life Sciences Center 9900 Blackwell Road

Rockville, Maryland 20850

Animal Facility: Microbiological Associates, Inc.

5221 River Road

Bethesda, Maryland 20816

#### I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate

Study Director: Janet Luczak, M.T. (ASCP),

M.G.A.

#### J. Schedule:

1. Study Initiation Date: 07/16/90

2. Lab Initiation Date: 08/03/90

3. Lab Completion Date: 08/31/90

4. Study Completion Date: See Study Director's Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

#### L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

#### III. PROCEDURES

#### A. Objective:

The study objective is to detect inapparent adventitious agent(s) which might be present in the test article, pre-clarified bulk live virus and/or fluids, other than the specific virus in the product.

#### B. Methods:

1. Test System Identification and Randomization

Each rabbit was housed individually. Each animal cage was assigned a number and labelied as "test article" or "uninoculated control". Each rabbit was identified by a unique number tattooed on it's ear. The rabbit's number was recorded on the cage card.

The rabbits were randomized according to SOP #OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. The rabbits were observed for 28 days for clinical signs of illness or distress.

# 3. Animal Husbandry

- a. Rabbits were fed certified rabbit chow <u>ad</u>
  <u>libitum</u> and water was supplied via water
  bottles, <u>ad</u> <u>libitum</u>.
- b. Rabbit's cages were changed weekly.
- c. Animal facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

#### IV. RESULTS

All rabbits inoculated with the test article and the uninoculated control rabbit remained normal and healthy for the 28 day observation period.

See Tables 2 and 3 for a summary of the data.

# V. CONCLUSIONS

No evidence of contamination with adventitious agent(s) due to the test article, Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7, was observed.

#### VI. **APPROVALS**

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice regulations as found in Title 21 CFR Part 58.

hileman

Mary D. Whiteman Study Director

9/27/90

# TABLE 1

Group	No. of Animals	Routes of Inoculation	Volume of Inoculum	Inoculum	Treatment after <u>Inoculation</u>
1	1	I.D. S.Q.	1.0 ml 9.0 ml	Test Article	Observe for Illness
2	1	SAA	SAA	SAA	SAA
3	1	SAA	SAA	SAA	SAA
4	1.	None	None	None	SAA

SAA = Same As Above

I.D. = Intradermal Inoculation

S.Q. = Subcutaneous Inoculation

TABLE 2 Survival Summary for Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7

	RABBITS	
Test Article	3/3 <sup>a</sup>	
Uninoculated Control	1/1 <sup>b</sup>	

a Number of surviving, healthy animals after 28 days/Number of

animals inoculated.

b Number of surviving, healthy animals after 28 days/Number of animals on lab initiation date.

Summary of Daily Observations for Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post- inoc.)	Day of Death/ Sacrifice (Post- inoc.)
Rabbit	Test Article	8 9 10	Normal Normal Normal		
	Uninoc- ulated Control	4	Normal		

# QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT RABBITS

Study Number: ZA358.005101

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/23/90 - 07/23/90, TO STUDY DIR 07/23/90, TO MGMT 07/23/90 PHASES: PROTOCOL REVIEW

INSPECT ON 08/16/90 - 08/16/90, TO STUDY DIR 08/16/90, TO MGMT 08/21/90 PHASES: ANIMAL OBSERVATIONS

INSPECT ON 09/21/90 - 09/24/90, TO STUDY DIR 09/24/90, TO MGMT 09/28/90 PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Ed Warburton Date
Quality Assurance Unit

# APPENDIX

С

# ANIMAL SAFETY TEST IN ADULT MICE AND SUCKLING MICE

Study NO.: ZA356.005100						
Dengue-1 Prod Seed: PDK-10,	FRhL-2/d7	• • • • • • • • • • • • • • • • • • • •	pages	55	-	67
Study NO.: ZA357.005100 Dengue-1 Prod Seed: PDK-20,	FRhL-2/d7	•••••	pages	68	-	80
Study NO.: ZA358.005100						
Dengue-1 Prod Seed: PDK-27,	FRhL-2/d7	• • • • • • • •	pages	81	_	93

# ANIMAL SAFETY TEST IN ADULT MICE AND SUCKLING MICE

Study No.: ZA356.005100

Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7

Final Report For

Program Resources, Inc. Biomedical Services Division 7655 Old Springhouse Road McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

# ZA356.005100

# TABLE OF CONTENTS

		Page
	Summary	57
I.	Introduction	58
II.	Study Information	58
III.	Procedures	60
IV.	Results	61
v.	Conclusions	62
VI.	Approvals	62
VII.	Quality Assurance Statement	. 67



#### SUMMARY

The purpose of this assay is to attempt to detect the presence of adventitious agent(s) in the test article, preclarified bulk live virus and/or fluids, other than the specific virus in the product. The test article was inoculated into adult mice and suckling mice. The suckling mouse portion of the assay included a subpassage of homogenized tissue, after 14 days, into a new group of suckling mice, followed by an additional 14 day observation period.

No evidence of viral contamination due to the test article Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7 was observed.

#### ZA356.005100

#### I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent virus(es). The experimental design utilizes inoculations of adult and suckling mice. The test is performed as described in CFR Title 21, Section 630.35 (a)(1)(2).

Adult mice are included in the assay to detect possible contamination of the test article with neurotropic or other viruses such as lymphocytic choriomeningitis virus. Suckling mice are used to detect Coxsackie or other viruses. All animals are observed for signs of illness and any that become sick or show any abnormalities are examined in an attempt to establish cause of illness or death.

#### II. STUDY INFORMATION

- A. Title: Animal Safety Test in Adult Mice and Suckling Mice
- B. Study Number: ZA356.005100
- C. Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7 was received at Microbiological Associates, Inc. on 07/06/90. The test article was received frozen. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.
- D. Medium Test Article: none
- E. Control Articles:
  - 1. Positive Control: none
  - 2. Negative Control: none
  - 3. Vehicle Control: none
- F. Test System: Mice

Suckling litters (Primary Inoculation): Tac:(SW)FBR, three adult females each with ten <24 hour old suckling pups,

Source: Taconic Farms
Germantown, New York

MICROBIOLOGICAL ASSOCIATES, INC.

#### ZA356.005100

Suckling litters (Blind Passage): Tac: (SW) FBR, four adult females each with ten <24 hour old suckling pups,

Source: Taconic Farms

Germantown, New York

Adult - HSD:ICR, Fifteen males and fifteen females, Body Weight range: 15-20 grams

Body Weight range: 15-20 grams.

Source: Harlan Sprague Dawley, Frederick, Maryland

G. Sponsor: Program Resources, Inc.

Biomedical Services Division 7655 Old Springhouse Road

McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department

Microbiological Associates, Inc.

Life Sciences Center 9900 Blackwell Road

Rockville, Maryland 20850

Animal Facility: 5221 River Road

Bethesda, Maryland 20816

#### I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate

Study Director: Janet Luczak, M.T. (ASCP), M.G.A.

#### J. Schedule:

1. Study Initiation Date: 07/16/90

2. Lab Initiation Date: 07/24/90

3. Lab Completion Date: 08/23/90

4. Study Completion Date: See Study Director's

Signature Date, in the

"Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.



# L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

#### III. PROCEDURES

With approval of the sponsor, a previously thawed sample which was frozen back and stored at -70°C was utilized in the inoculation of the suckling mouse portion of the assay on 07/24/90. In addition, at the request of the sponsor, in the suckling mouse portion of the assay, 1.3 ml of the test article was combined with 1.3 ml of sponsor supplied antisera Den-1 Jamaica HMAF, 4-17-78 and heated at 37°C for 90 minutes, prior to inoculation of the suckling mice. The remaining untreated sample was again frozen back and was utilized along with a previously unthawed aliquot of the test article to inoculate the adult mouse portion of the assay on 07/26/90.

# A. Objective:

The study objective is to detect inapparent virus(es) that might be present in the test article, preclarified bulk live virus and/or fluids, other than the specific virus in the product.

# B. Methods:

1. Test System Identification and Randomization

Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Adult mice were ear-tagged but housed in groups according to inoculum type and sex. Suckling mice were not individually identified.

Suckling and adult mice were randomized according to SOP #OPBT0213.

#### 2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. The adult mice were observed every working day for 28 days for clinical signs. In the suckling mouse portion of the assay, the animals were inoculated



according to Table 1 and were then observed every working day for 14 days for clinical signs. Fourteen days post-inoculation, all surviving suckling mice from each group were euthanized using cervial dislocation. Following euthanasia their skin and gastrointestine were removed, the carcasses cut into pieces and placed in a sterile pre-weighed bowl. After determining the weight of the entire group of mice from a cage, enough Hank's Balanced Salt Solution (HBSS) with gentamicin was added to make a 20% w/v suspension. The entire content of the bowl was then homogenized in a sterile blender, clarified by centrifugation, diluted 1:2 in HBSS and subsequently inoculated into a new group of suckling mice by the same routes and in the same volumes as the original group. These newly inoculated mice were observed for a period of fourteen days. Ten suckling mice were held as uninoculated controls and observed for fourteen days.

# 3. Animal Husbandry

a. All animals were fed the following diet ad libitum:

Mice - autoclavable chow.

- b. Water was supplied ad libitum via fresh apples (disinfected).
- c. Bedding Bed-o-Cobs, Anderson Cob Mills.
  Cages were changed as necessary, usually twice per week.
- d. Animal facilities are accredited by the American Association for Accreditation of Laboratory Animal Care.

#### IV. RESULTS

All adult mice inoculated with the test article and all uninoculated control adult mice remained normal and healthy for the twenty-eight day observation period.

All uninoculated control suckling mice and all test article inoculated suckling mice appeared normal and healthy for the 14 day observation period. The surviving mice of each group were homogenized and the homogenate of each group was passaged into a new group of suckling mice. The remainder of the homogenates was frozen at -70°C.



In the blind passage, all of the uninoculated control suckling mice, all of the suckling mice inoculated with the homogenate of the uninoculated control suckling mice and all of the suckling mice inoculated with the homogenate of the test article inoculated suckling mice appeared normal and healthy for the 14 day observation period.

See Tables 2 and 3 for a summary of the data.

#### V. CONCLUSIONS

No evidence of viral contamination due to the test article, Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7, was observed.

#### VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman

Study Wirector

Data

TABLE 1

Group #	Number of Animals	Sex	Species	Route(s) of Inoculation	Volume of   Inoculum	Inoculum	Treatment after Inoculation,
SM 1	1	female	mouse (lactating)	None.	None	None	None
	+ 10	various	Mouse (suckling)	î.p. i.c.	0.1 ml 0.01 ml	test article test article	Observed for illness after 14 days passage a single pool of emulsified tissue (minus skin and gastrointestine) of all surviving mice onto at least 10 additional suckling mice. Use same routes and volumes as original.
SM 2	SAA*	SAA	SAA	SAA	SAA	SAA	SAA
SM 3	SAA	SAA	SAA	Нопе	None	uninoc control	SAA
AM 1	5	male	mouse	i.p. i.c.	0.5 ml 0.03 ml	test article	Observe for illness
AM 2	5	male	SAA	SAA	SAA	SAA	SAA
AM 3	5	female	SAA	SAA	SAA	SAA	SAA
AM 4	5	female	SAA	SAA	SAA	SAA	SAA
AM 5	5	male	SAA	None	None	uninoc control	SAA
AM 6	5	female	SAA	None	None	SAA	SAA

<sup>\*</sup>SAA = Same as above



i.c. = Intracranial

i.p. = Intraperitoneal

TABLE 2

Survival Summary
for Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7

		Suckling N	lice <sup>b</sup>	
	Adult Mice <sup>a</sup>	Primary Inoculation	Blind Passage	
Test Article	20/20	20/20	20/20	
Uninoculated Control	10/10	10/10	10/10	
Uninoculated Control <sup>C</sup>			10/10	

a Number of surviving, healthy animals after 28 days/Number of animals inoculated.



b In the suckling mice portion of the assay, animals are inoculated and observed for 14 days. On day 14 post-inoculation a homogenate is prepared from the surviving sucklings from each group. This homogenate was used to inoculate another group of suckling mice which were observed for an additional 14 days. The number of surviving sucklings/number of animals inoculated is presented.

<sup>&</sup>lt;sup>C</sup> In the blind passage of the suckling mouse portion of the assay an uninoculated control group was held with the blind passage animals inoculated with the homogenate of the test article and the homogenate of the uninoculated control group from the primary inoculation.

TABLE 3

Summary of Daily Observations
for Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post- inoc.)	Day of Death/ Sacrifice (Post- inoc.
Adult Mice	Test Article	10501 10502 10503 10504 10505 10506 10507 10508 10509 10510 10511 10512 10513 10514 10515 10516 10517 10518 10519 10520	Normal		
	Uninoc- ulated Control	10521 10522 10523 10524 10525 10526 10527 10528 10529 10530	Normal Normal Normal Normal Normal Normal Normal Normal Normal		

TABLE 3 (Cont.)

# Summary of Daily Observations for Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7

						-
•	Inoculum	Numb Cage		Clinical Signs	Day of Onset (Post- Inoc.)	Day of Death/ Sacrifice (Post- Inoc.)
Suckling <sup>b</sup> Mice						
(Primary Inoculation)	Test Article		(10) (10)	Normal Normal		
	Uninocu- lated Control	SM3	(10)	Normal		
(Blind Passage)	Test Article Homo- genate		(10) (10)	Normal Normal		
	Uninocu- lated Control Homo- genate	SM3	(10)	Normal		
	Uninocu- lated Control	SM4	(10)	Normal		

Ten suckling mice inoculated per cage.

b Surviving suckling mice from primary inoculation were sacrificed on day 14 for preparation of blind passage tissue homogenate.

#### QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT MICE AND SUCKLING MICE

Study Number: ZA356.005100

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPS (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/19/90 - 07/19/90, TO STUDY DIR 07/19/90, TO MGMT 07/19/90 PHASES: PROTOCOL REVIEW

INSPECT ON 08/23/90 - 08/23/90, TO STUDY DIR 08/23/90, TO MGMT 08/23/90 PHASES: OBSERVATION OF ANIMALS FOR CLINICAL SIGNS

INSPECT ON 08/30/90 - 08/30/90, TO STUDY DIR 08/30/90, TO MGMT 08/31/90 PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Joan M. McGowan S/31/90

Quality Assurance Unit

# ANIMAL SAFETY TEST IN ADULT MICE AND SUCKLING MICE

Study No.: ZA357.005100

Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7

Final Report For

Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

# ZA357.005100

## TABLE OF CONTENTS

		Page
	Summary	70
I.	Introduction	71
II.	Study Information	71
III.	Procedures	73
IV.	Results	74
v.	Conclusions	75 <sup>-</sup>
VI.	Approvals	75
VII.	Quality Assurance Statement	80

#### SUMMARY

The purpose of this assay is to attempt to detect the presence of adventitious agent(s) in the test article, preclarified bulk live virus and/or fluids, other than the specific virus in the product. The test article was inoculated into adult mice and suckling mice. The suckling mouse portion of the assay included a subpassage of homogenized tissue, after 14 days, into a new group of suckling mice, followed by an additional 14 day observation period.

No evidence of viral contamination due to the test article Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7 was observed.

#### ZA357.005100

#### I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent virus(es). The experimental design utilizes inoculations of adult and suckling mice. The test is performed as described in CFR Title 21, Section 630.35 (a)(1)(2).

Adult mice are included in the assay to detect possible contamination of the test article with neurotropic or other viruses such as lymphocytic choriomeningitis virus. Suckling mice are used to detect Coxsackie or other viruses. All animals are observed for signs of illness and any that become sick or show any abnormalities are examined in an attempt to establish cause of illness or death.

#### II. STUDY INFORMATION

- A. Title: Animal Safety Test in Adult Mice and Suckling Mice
- B. Study Number: ZA357.005100
- C. Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7 was received at Microbiological Associates, Inc. on 07/06/90. The test article was received frozen. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.
- D. Medium Test Article: none
- E. Control Articles:
  - 1. Positive Control: none
  - 2. Negative Control: none
  - 3. Vehicle Control: none
- F. Test System: Mice

Suckling litters (Primary Inoculation): Tac:(SW)FBR, three adult females each with ten <24 hour old suckling pups,

Source: Taconic Farms

Germantown, New York

Suckling litters (Blind Passage): Tac:(SW)FBR, four adult females each with ten <24 hour old suckling pups,

Source: Taconic Farms

Germantown, New York

Adult - HSD:ICR, Fifteen males and fifteen females, Body Weight range: 15-20 grams.

Source: Harlan Sprague Dawley, Frederick, Maryland

G. Sponsor: Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

Animal Facility: 5221 River Road

Bethesda, Maryland 20816

#### I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate
Study Director: Janet Luczak, M.T. (ASCP), M.G.A.

#### J. Schedule:

1. Study Initiation Date: 07/16/90

2. Lab Initiation Date: 07/24/90

3. Lab Completion Date: 08/23/90

4. Study Completion Date: See Study Director's Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

#### ZA357.005100

#### L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

#### III. PROCEDURES

ø

With approval of the sponsor, a previously thawed sample which was frozen back and stored at -70°C was utilized in the inoculation of the suckling mouse portion of the assay on 07/24/90. In addition, at the request of the sponsor, in the suckling mouse portion of the assay, 1.3 ml of the test article was combined with 1.3 ml of sponsor supplied antisera Den-1 Jamaica HMAF, 4-17-78 and heated at 37°C for 90 minutes, prior to inoculation of the suckling mice. The remaining untreated sample was again frozen back and was utilized along with a previously unthawed aliquot of the test article to inoculate the adult mouse portion of the assay on 07/26/90.

## A. Objective:

The study objective is to detect inapparent virus(es) that might be present in the test article, pre-clarified bulk live virus and/or fluids, other than the specific virus in the product.

#### B. Methods:

1. Test System Identification and Randomization

Each a imal cage was assigned a number and labelled as "test article" or "uninoculated control". Adult mice were ear-tagged but housed in groups according to inoculum type and sex. Suckling mice were not individually identified.

Suckling and adult mice were randomized according to SOP #OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. The adult mice were observed every working day for 28 days for clinical signs. In the suckling mouse portion of the assay, the animals were inoculated according to Table 1 and were then observed every

working day for 14 days for clinical signs. Fourteen days post-inoculation, all surviving suckling mice from each group were euthanized using cervical dislocation. Following euthanasia their skin and gastrointestine were removed, the carcasses cut into pieces and placed in a sterile pre-weighed bowl. After determining the weight of the entire group of mice from a cage, enough Hank's Balanced Salt Solution (HBSS) with gentamicin was added to make a 20% w/v suspension. The entire content of the bowl was then homogenized in a sterile blender, clarified by centrifugation, diluted 1:2 in HBSS and subsequently inoculated into a new group of suckling mice by the same routes and in the same volumes as the original group. These newly inoculated mice were observed for a period of fourteen days. Ten suckling mice were held as uninoculated controls and observed for fourteen days.

## 3. Animal Husbandry

a. All animals were fed the following diet ad libitum:

Mice - autoclavable chow.

- b. Water was supplied ad libitum via fresh apples (disinfected).
- c. Bedding Bed-o-Cobs, Anderson Cob Mills. Cages were changed as necessary, usually twice per week.
- d. Animal facilities are accredited by the American Association for Accreditation of Laboratory Animal Care.

#### IV. RESULTS

All adult mice inoculated with the test article and all uninoculated control adult mice remained normal and healthy for the twenty-eight day observation period.

All uninoculated control suckling mice and test article inoculated suckling mice appeared normal and healthy for the 14 day observation period. The surviving mice of each group were homogenized and the homogenate of each group was passaged into a new group of suckling mice. The remainder of the homogenates was frozen at -70°C.



#### ZA357.005100

In the blind passage all of the uninoculated control suckling mice, all of the suckling mice inoculated with the homogenate of the uninoculated control suckling mice, and nineteen of the twenty suckling mice inoculated with the homogenate of the test article inoculated suckling mice appeared normal and healthy for the 14 day observation period. One of the test article homogenate inoculated suckling mice was missing and presumed cannibalized day 3 post-inoculation.

See Tables 2 and 3 for a summary of the data.

#### V. CONCLUSIONS

No evidence of viral contamination due to the test article, Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7, was observed.

#### VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman

Study Director

Date

TABLE 1

Group #	Number of Animals	Sex	Species [	Route(s) of Inoculation	Volume of   Inoculum	Inoculum	Treatment after Inoculation
SM 1	1	female	mouse (lactating)	None	None	None	None
	+ 10	various	Mouse (suckling)	i.p. i.c.	0.1 ml 0.01 ml	test article test article	Observed for illness after 14 days passage a single pool of emulsified tissue (minus skin and gastrointestine) of all surviving mice onto at least 10 additional suckling mice. Use same routes and volumes as original.
SM 2	SAA*	SAA	SAA	SAA	SAA	SAA	SAA
SM 3	SAA	SAA	SAA	None	None	uninoc control	SAA .
AM 1	5	male	mouse	i.p. i.c.	0.5 ml 0.03 ml	test article	Observe for illness
AH 2	5	male	SAA	SAA	SAA	SAA	SAA
AM 3	5	female	SAA	SAA	SAA	SAA	SAA
AM 4	5	female	SAA	SAA	SAA	SAA	SAA
AM 5	5	male	SAA	None	None	uninoc control	SAA
AM 6	5	female	SAA	None	None	SAA	SAA

<sup>\*</sup>SAA = Same as above

i.c. = Intracranial

i.p. = Intraperitoneal

TABLE 2

Survival Summary

for Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7

		Suckling Miceb		
	Adult Mice <sup>a</sup>	Primary Inoculation	Blind Passage	
Test Article	20/20	20/20	19/20	
Uninoculated Control	10/10	10/10	10/10	
Uninoculated Control <sup>C</sup>			10/10	

a Number of surviving, healthy animals after 28 days/Number of animals inoculated.

b In the suckling mice portion of the assay, animals are inoculated and observed for 14 days. On day 14 post-inoculation a homogenate is prepared from the surviving sucklings from each group. This homogenate was used to inoculate another group of suckling mice which were observed for an additional 14 days. The number of surviving sucklings/number of animals inoculated is presented.

<sup>&</sup>lt;sup>C</sup> In the blind passage of the suckling mouse portion of the assay an uninoculated control group was held with the blind passage animals inoculated with the homogenate of the test article and the homogenate of the uninoculated control group from the primary inoculation.

TABLE 3

Summary of Daily Observations
for Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post- inoc.)	Day of Death/ Sacrifice (Post- inoc.
Adult Mice	Test Article	10531 10532 10533 10534 10535 10536 10537 10538 10539 10540 10541 10542 10543 10544 10545 10546 10547 10548 10549 10550	Normal		
	Uninoc- ulated Control	10521 10522 10523 10524 10525 10526 10527 10528 10529 10530	Normal		

TABLE 3 (Cont.) Summary of Daily Observations for Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7

Animal Species	Inoculum	Numb Cage		Clinical Signs	Day of Onset (Post- Inoc.)	Day of Death/ Sacrifice (Post- Inoc.)
Suckling <sup>b</sup> Mice						
(Primary Inoculation)	Test Article		(10) (10)	Normal Normal		
	Uninocu- lated Control	SM3	(10)	Normal		
(Blind Passage)	Test Article Homo- genate	SM1 SM2	(10) (9) <sup>C</sup> (10)	Normal - Normal	3	3
	Uninocu- lated Control Homo- genate	SM3	(10)	Normal		
	Uninocu- lated Control	SM4	(10)	Normal		



a Ten suckling mice inoculated per cage.

b Surviving suckling mice from primary inoculation were sacrificed on day 14 for preparation of blind passage tissue homogenate.

C One suckling mouse missing and presumed cannibalized.

# QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT MICE AND SUCKLING MICE

Study Number: ZA357.005100

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/19/90 - 07/19/90, TO STUDY DIR 07/19/90, TO MGMT 07/19/90 PHASES: PROTOCOL REVIEW

INSPECT ON 08/23/90 - 08/23/90, TO STUDY DIR 08/23/90, TO MGMT 08/23/90 PHASES: OBSERVATION OF ANIMALS FOR CLINICAL SIGNS

INSPECT ON 08/30/90 - 08/30/90, TO STUDY DIR 08/30/90, TO MGMT 08/31/90 PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Joan M. McGowan

Quality Assurance Unit

MICROBIOLOGICAL ASSOCIATES, INC.

# ANIMAL SAFETY TEST IN ADULT MICE AND SUCKLING MICE

Study No.: ZA358.005100

Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7

Final Report For

Program Resources, Inc. Biomedical Services Division 7655 Old Springhouse Road McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

# TABLE OF CONTENTS

		Page
	Summary	83
I.	Introduction	84
II.	Study Information	84
III.	Procedures	86
IV.	Results	87
v.	Conclusions	88
VI.	Approvals	88
VII.	Quality Assurance Statement	93

#### SUMMARY

The purpose of this assay is to attempt to detect the presence of adventitious agent(s) in the test article, preclarified bulk live virus and/or fluids, other than the specific virus in the product. The test article was inoculated into adult mice and suckling mice. The suckling mouse portion of the assay included a subpassage of homogenized tissue, after 14 days, into a new group of suckling mice, followed by an additional 14 day observation period.

No evidence of viral contamination due to the test article Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7 was observed.

#### ZA358.005100

#### I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent virus(es). The experimental design utilizes inoculations of adult and suckling mice. The test is performed as described in CFR Title 21, Section 630.35 (a)(1)(2).

Adult mice are included in the assay to detect possible contaminat on of the test article with neurotropic or other viruses such as lymphocytic choriomeningitis virus. Suckling mice are used to detect Coxsackie or other viruses. All animals are observed for signs of illness and any that become sick or show any abnormalities are examined in an attempt to establish cause of illness or death.

#### II. STUDY INFORMATION

- A. Title: Animal Safety Test in Adult Mice and Suckling Mice
- B. Study Number: ZA358.005100
- C. Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7 was received at Microbiological Associates, Inc. on 07/06/90. The test article was received frozen. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.
- D. Medium Test Article: none
- E. Control Articles:
  - 1. Positive Control: none
  - 2. Negative Control: none
  - 3. Vehicle Control: none
- F. Test System: Mice

Suckling litters (Primary Inoculation): Tac:(SW)FBR, three adult females each with ten <24 hour old suckling pups,

Source: Taconic Farms

Germantown, New York

Suckling litters (Blind Passage): Tac:(SW)FBR, four adult females each with ten <24 hour old suckling pups,

Source: Taconic Farms

Germantown, New York

Adult - HSD:ICR, Fifteen males and fifteen females, Body Weight range: 15-20 grams. Source: Harlan Sprague Dawley, Frederick, Maryland

G. Sponsor: Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department Microbiological Associates, Inc. Life Sciences Center 9900 Blackwell Road Rockville, Maryland 20850

Animal Facility: 5221 River Road

Bethesda, Maryland 20816

#### I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate Study Director: Janet Luczak, M.T. (ASCP), M.G.A.

# J. Schedule:

1. Study Initiation Date: 07/16/90

2. Lab Initiation Date: 07/24/90

3. Lab Completion Date: 08/23/90

4. Study Completion Date: See Study Director's Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.



#### L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

#### III. PROCEDURES

With approval of the sponsor, a previously thawed sample which was frozen back and stored at -70°C was utilized in the inoculation of the suckling mouse portion of the assay on 07/24/90. In addition, at the request of the sponsor, in the suckling mouse portion of the assay, 1.3 ml of the test article was combined with 1.3 ml of sponsor supplied antisera Den-1 Jamaica HMAF, 4-17-78 and heated at 37°C for 90 minutes, prior to inoculation of the suckling mice. The remaining untreated sample was again frozen back and was utilized along with a previously unthawed aliquot of the test article to inoculate the adult mouse portion of the assay on 07/26/90.

### A. Objective:

The study objective is to detect inapparent virus(es) that might be present in the test article, preclarified bulk live virus and/or fluids, other than the specific virus in the product.

#### B. Methods:

1. Test System Identification and Randomization

Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Adult mice were ear-tagged but housed in groups according to inoculum type and sex. Suckling mice were not individually identified.

Suckling and adult mice were randomized according to SOP #OPBT0213.

#### 2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. The adult mice were observed every working day for 28 days for clinical signs. In the suckling mouse portion of the assay, the animals were inoculated according to Table 1 and were then observed every working day for 14 days for clinical signs.



Fourteen days post-inoculation, all surviving suckling mice from each group were euthanized using cervical dislocation. Following euthanasia their skin and gastrointestine were removed, the carcasses cut into pieces and placed in a sterile pre-weighed bowl. After determining the weight of the entire group of mice from a cage, enough Hank's Balanced Salt Solution (HBSS) with gentamicin was added to make a 20% w/v suspension. The entire content of the bowl was then homogenized in a sterile blender, clarified by centrifugation, diluted 1:2 in HBSS and subsequently inoculated into a new group of suckling mice by the same routes and in the same volumes as the original group. These newly inoculated mice were observed for a period of fourteen days. Ten suckling mice were held as uninoculated controls and observed for fourteen days.

#### 3. Animal Husbandry

a. All animals were fed the following diet ad libitum:

Mice - autoclavable chow.

- Water was supplied ad libitum via fresh apples (disinfected).
- c. Bedding Bed-o-Cobs, Anderson Cob Mills. Cages were changed as necessary, usually twice per week.
- d. Animal facilities are accredited by the American Association for Accreditation of Laboratory Animal Care.

#### IV. RESULTS

All adult mice inoculated with the test article and all uninoculated control adult mice remained normal and healthy for the twenty-eight day observation period.

All uninoculated control suckling mice and all test article inoculated suckling mice appeared normal and healthy for the 14 day observation period. The surviving mice of each group were homogenized and the homogenate of each group was passaged into a new group of suckling mice. The remainder of the homogenates was frozen at -70°C.



#### ZA358.005100

In the blind passage, all of the uninoculated control suckling mice, all of the suckling mice inoculated with the homogenate of the uninoculated control suckling mice and all of the suckling mice inoculated with the homogenate of the test article inoculated suckling mice appeared normal and healthy for the 14 day observation period.

See Tables 2 and 3 for a summary of the data.

#### V. CONCLUSIONS

No evidence of viral contamination due to the test article, Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7, was observed.

#### VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman

Study Director

Date

TABLE 1

Group #	Number of Animals	Sex	Species	Route(s) of Inoculation	Volume of   Inoculum	Inoculum	Treatment after inoculation
SM 1	1	female	mouse (lactating)	None	None	None	None
	+						
	10	various	Mouse	i.p.	0.1 ml	test article	Observed for illness after 14
			(suckling)	i.c.	0.01 ml	test article	days passage a single pool of emulsified tissue (minus skin and gastrointestine) of all surviving mice onto at least 10 additional suckling mice. Use same routes and volumes as original
SM 2	SAA*	SAA	SAA	SAA	SAA	SAA	SAA
SM 3	SAA	SAA	SAA	None	None	uninoc control	saa
AM 1	5	male	mouse	i.p.	0.5 ml 0.03 ml	test article	Observe for illness
AM 2	5	male	SAA	SAA	SAA	SAA	SAA
AH 3	5	female	SAA	SAA	SAA	SAA	SAA
AM 4	5	female	SAA	SAA	SAA	SAA	SAA
AM 5	5	male	SAA	None	None	uninoc control	SAA
AM 6	5	female	SAA	None	None	SAA	SAA

\*SAA = Same as above

i.c. = Intracranial

i.p. = Intraperitoneal



TABLE 2

Survival Summary

for Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7

		Suckling Miceb				
	Adult Mice <sup>a</sup>	Primary Inoculation	Blind Passage			
Test Article	20/20	20/20	20/20			
Uninoculated Control	10/10	10/10	10/10			
Uninoculated Control <sup>C</sup>			10/10			

a Number of surviving, healthy animals after 28 days/Number of animals inoculated.



b In the suckling mice portion of the assay, animals are inoculated and observed for 14 days. On day 14 post-inoculation a homogenate is prepared from the surviving sucklings from each group. This homogenate was used to inoculate another group of suckling mice which were observed for an additional 14 days. The number of surviving sucklings/number of animals inoculated is presented.

C In the blind passage of the suckling mouse portion of the assay, an uninoculated control group was held with the blind passage animals inoculated with the homogenate of the test article and the homogenate of the uninoculated control group from the primary inoculation.

TABLE 3

Summary of Daily Observations
for Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post- inoc.)	Day of Death/ Sacrifice (Post- inoc.
Adult Mice	Test Article	10551 10552 10553 10554 10555 10556 10557 10558 10559 10560 10561 10562 10563 10564 10565 10566 10567 10568 10569 10570	Normal		
	Uninoc- ulated Control	10521 10522 10523 10524 10525 10526 10527 10528 10529 10530	Normal Normal Normal Normal Normal Normal Normal Normal Normal		

TABLE 3 (Cont.)

# Summary of Daily Observations for Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7

						-
Animal Species I	Inoculum	Numb Cage		Clinical Signs	Day of Onset (Post- Inoc.)	Day of Death/ Sacrifice (Post- Inoc.)
Suckling <sup>b</sup> Mice	· · · · · · · · · · · · · · · · · · ·					
(Primary Inoculation)	Test Article		(10) (10)	Normal Normal		
	Uninocu- lated Control	SM3	(10)	Normal		
(Blind Passage)	Test Article Homo- genate		(10) (10)	Normal Normal		
	Uninocu- lated Control Homo- genate	SM3	(10)	Normal		
	Uninocu- lated Control	SM4	(10)	Normal		

Ten suckling mice inoculated per cage.

b Surviving suckling mice from primary inoculation were sacrificed on day 14 for preparation of blind passage tissue homogenate.

## QUALITY ASSURANCE STAPEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT MICE AND SUCKLING MICE

Study Number: ZA358.005100

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/19/90 - 07/19/90, TO STUDY DIR 07/19/90, TO MGMT 07/19/90 PHASES: PROTOCOL REVIEW

INSPECT ON 08/23/90 - 08/23/90, TO STUDY DIR 08/23/90, TO MGMT 08/21/90 PHASES: OBSERVATION OF ANIMALS FOR CLINICAL SIGNS

INSPECT ON 08/30/90 - 08/30/90, TO STUDY DIR 08/30/90, TO MGMT 08/31/90 PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Joan M. McGowan Date
Quality Assurance Unit

# APPENDIX

D

# ANIMAL SAFETY TEST IN ADULT GUINEA PIGS

Study NO.: ZA356.005102	
Dengue-1 Prod Seed: PDK-10, FRhL-2	/d7 95 - 108
Study NO.: ZA357.005½02 Dengue-1 Prod Seed: PDK-20, FRhL-2	./d7 109 - 122
Study NO.: ZA358.005102 Dengue-1 Prod Seed: PDK-27. FRbL-2	2/d7 123 - 136

# ANIMAL SAFETY TEST IN ADULT GUINEA PIGS

Study No.: ZA356.005102

Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7

Final Report

Program Resources, Inc. Biomedical Services Division 7655 Old Springhouse Road McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

# TABLE OF CONTENTS

		Page
	Summary	97
I.	Introduction	98
II.	Study Information	98
III.	Procedures	99
IV.	Results	102
v.	Conclusions	102
·vI.	Approvals	102
VII.	Quality Assurance Statement	104
VIII.	Appendix	105

#### SUMMARY

The purpose of this test is to detect the presence of adventitious agent(s) which might be present in the test article, preclarified bulk live virus and/or fluids, other than the specific virus in the product, by the inoculation and observation of guinea pigs.

No evidence of contamination with adventitious agents was observed due to the test article Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7.

#### ZA356,005102

#### I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent adventitious agent(s). The experimental design utilizes the inoculation of adult guinea pigs. The test is performed as described in CFR Title 21, Section 630.16(a)(4).

Adult guinea pigs are used in this assay to detect possible contamination of the test article with M. tuberculosis or other adventitious agent(s). Animals are examined for signs of illness, and any that become sick or show any abnormalities are examined in an attempt to determine the cause of illness or death.

#### II. STUDY INFORMATION

- A. Title: Animal Safety Test in Adult Guinea Pigs
- B. Study Number: ZA356.005102
- C. Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7 was received at Microbiological Associates, Inc. on 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.
- D. Medium Test Article: none
- E. Control Articles:
  - 1. Positive Control: none
  - 2. Negative Control: none
  - 3. Vehicle Control: none
- F. Test System: Guinea Pigs

Hartley Albino 6 adult females,

Body weight range: 350-400 g

Source: Hazleton Research Animals

Denver, Pennsylvania

G. Sponsor: Program Resources, Inc.

Biomedical Services Division 7655 Old Springhouse Road

McLean, VA 22102

Authorized Representative: Dr. Louis Potash

#### ZA356.005102

H. Testing Facility: Biotechnology Services Department

Microbiological Associates, Inc.

Life Sciences Center 9900 Blackwell Road

Rockville, Maryland 20850

Animal Facility: Microbiological Associates, Inc.

5221 River Road

Bethesda, Maryland 20816

#### I. Personnel:

1. Study Director: Mary D. Whiteman

 Associate Study Director: Janet Luczak, M.T. (ASCP), M.G.A.

#### J. Schedule:

Study Initiation Date: 07/11/90

2. Lab Initiation Date: 07/12/90

3. Lab Completion Date: 09/06/90

4. Study Completion Date: See Study Director's

Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

## L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

#### III. PROCEDURES

### A. Objective:

The study objective is to detect adventitious agent(s) that might be present in the test article.

#### ZA356.005102

#### B. Methods:

1. Test System Identification and Randomization

Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Animals were housed separately and were identified by ear tags.

The guinea pigs were randomized according to SOP OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. Three were held as uninoculated controls. All animals were observed every working day of the 42 day test period for death or clinical signs of illness or distress. Beginning day 21 post inoculation, rectal temperatures were recorded through day 42 post-inoculation. All remaining guinea pigs were sacrificed. Gross pathological examinations were performed on all guinea pigs.

#### 3. Animal Husbandry

- a. Animals were fed Ralston Purina Certified Guinea Pig Chow.
- b. Water was supplied <u>ad libitum</u> via water bottles. Water was disinfected with 7 ppm chlorine.
- c. Bedding Bed-o-Cobs, Anderson Cob Mills. Cages were changed as necessary, usually twice per week.
- d. Animal facilities are accredited by the American Association for Accreditation of Laboratory Animal Care.

TABLE 1

Group #	No. of Animals	Routes of Inoculation	Volume of Inoculum	Inoculum	Treatment after Inoculation
1	1.	I.C. I.P.	0.1 ml 5.0 ml	Test Article	Observe for Illness Record Rectal Temp. On Days 21-42 Post- Inoculation
2	1	SAA	SAA	SAA	SAA
3	1	SAA	SAA	SAA	SAA
4	1	None	None	Uninoc Control	SAA
5	1	SAA	SAA	SAA	SAA
6	1.	SAA	SAA	SAA	SAA

SAA = Same As Above
I.C. = Intracranial
I.P. = Intraperitoneal

#### ZA356.005102

#### IV. RESULTS

All of the uninoculated control guinea pigs and two of the three test article inoculated guinea pigs appeared normal and healthy throughout the 42 day observation period. One of the test article inoculated guinea pigs (#10401) was noted to have a noticeably decreased amount of feces production on day 15 post-inoculation, but appeared otherwise normal. By day 19 post-inoculation, feces production appeared normal and the animal remained normal and healthy for the duration of the 42 day observation period. See Table 2 for a summary of the data.

None of the uninoculated control quinea pigs and none of the test article inoculated quinea pigs had significant temperature rises indicative of either viral or bacterial infections during the 21 day recording period from day 21 through 42 post-inoculation. See Table 2 for a summary of the data.

At examination on day 42 for gross pathology, no lesions were found in the control or test article quinea pigs. (See Pathology report in Appendix.)

#### V. CONCLUSIONS

No evidence of contamination with adventitious agents due to the test article, Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7, was observed.

#### VI. **APPROVALS**

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice regulations as found in Title 21 CFR Part 58.

Whitemon Mary D. Whiteman Study Director



TABLE 2

Summary of Daily Observations
for Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post- inoc.)	Day of Death/ Sacrifice (Post- inoc.	Range of Body Temp in <sup>O</sup> C D-21 to D-42
Guinea Pig	Test Article	10401 10402 10403	_a Normal Normal	15	3	88.0 - 38.5 87.9 - 38.7 87.9 - 38.4
	Uninoc- ulated Control	10404 10405 10406	Normal Normal Normal		3	88.0 - 38.5 88.2 - 39.0 88.0 - 38.9

<sup>&</sup>lt;sup>a</sup> Animal was noted to have reduced feces production, but appeared otherwise normal. Feces production was normal by day 19 and appeared normal for the duration of the 42 day observation period.

#### QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT GUINEA PIGS

Study Number: ZA356.005102

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/16/90 - 07/16/90, TO STUDY DIR 07/16/90, TO MGMT 07/16/90 PHASES: PROTOCOL REVIEW

INSPECT ON 08/23/90 - 08/23/90, TO STUDY DIR 08/23/90, TO MGMT 08/23/90 PHASES: ANIMAL OBSERVATIONS

INSPECT ON 09/12/90 - 09/12/90, TO STUDY DIR 09/12/90, TO MGMT 09/14/90 PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Joan M. McGowan 9/14/90

Joan M. McGowan Date
Quality Assurance Unit

VIII. APPENDIX

CORPORATE OFFICES
Life Sciences Center
9900 Blackwell Road • Rockville • Maryland 20850
(301) 738-1000 • Telex 908793 • Fax (301) 738-1036

BETHESDA LABORATORIES 5221 River Road • Bethesda • Maryland 20816 (301) 654-3400 • Telex 90-8793

### PATHOLOGY REPORT

CAHS-2190		DANT BROWN	<b>VED:</b> 08/23/90
	Biotech Services ZA356.005102 ZA357.005102		•
	ZA358.005102 ZA359.005102 ZA360.005102 ZA361.005102		TED: 09/06/90
SPECIES:	Guinea Pig Results of gross examination	according	to SOP #865.201.
	ZA356.005102		
	2190-1 (10401) Test A Gross: No lesions found	rticle	563.9 ਖ਼ੁ
	2190-2 (10402) Test A Gross: No lesions found	rticle	684.2 g
	2190-3 (10403) Test A Gross: No lesions found	rticle	614.8 g
	2190-4 (10404) Contro Gross: No lesions found	1	614.4 g
	2190-5 (10405) Contro Gross: No lesions found	1	673.3 g
	2190-6 (10406) Contro Gross: No lesions found	1	646.2 g
	ZA357.005102		
	2190-7 (10407) Test A Gross: No lesions found	rticle	629.0 g
	2190-8 (10408) Test A Gross: No lesions found	rticle	583.6 g
	2190-9 (10409) Test A Gross: No lesions found	rticle	528.1 g

## PATHOLOGY REPORT

CAHS-2190

SOURCE: Biotech Services

PAGE 2

## ZA358.005102

2190-10 (10410) Test Article Gross: No lesions found	636.3 g
2190-11 (10411) Test Article Gross: Retroperitoneal abscess (4x6 peritonitis	490.5 g cm) and
2190-12 (10412)	595.5 g
ZA359.005102	
2190-13 (10421) Test Article Gross: No lesions found	605.3 g
2190-14 (10422) Test Article Gross: No lesions found	631.8 g
2190-15 (10423) Test Article Gross: No lesions found	547.4 g
2190-16 (10424) Control Gross: No lesions fo ad	651.5 g
2190-17 (10425) Control Gross: No lesions found	623.6 g
2190-18 (10426) Control Gross: No lesions found	615.9 g
ZA360.005102	
2190-19 (10427) Test Article Gross: No lesions found	561.8 g
2190-20 (10428) Test Article Gross: No lesions found	594.6 g
2190-21 (10429) Test Article Gross: No lesions found	598.4 g

PATHOLOGY REPORT

CAHS-2190

SOURCE: Biotech Services

PAGE 3

ZA361.005102

2190-22 (10430) Gross: No lesions	Article	559.8	g
2190-23 (10431) Gross: No lesions	Article	547.9	g

Test Article 624.8 g 2190-24 (10432) Gross: No lesions found

COMMENT: The retroperitoneal abscess in guinea pig #2190-11 is believed to be due to a rectal perforation (no longer visible) caused by insertion of a temperature measuring probe. This is a common lesion in guinea pigs in which the probe is inserted on multiple occasions.

Anton M. Allen, DVM, Ph.D.

Director of Veterinary Services

# ANIMAL SAFETY TEST IN ADULT GUINEA PIGS

Study No.: ZA357.005102

Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7

Final Report

Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

The state of the s

## TABLE OF CONTENTS

		Page
	Summary	111
I.	Introduction	112
II.	Study Information	112
III.	Procedures	113
IV.	Results	116
v.	Conclusions	116
VI.	Approvals	116
vII.	Quality Assurance Statement	118
VIII.	Appendix	119

#### SUMMARY

The purpose of this test is to detect the presence of adventitious agent(s) which might be present, in the test article, preclarified bulk live virus and/or fluids, other than the specific virus in the product, by the inoculation and observation of guinea pigs.

No evidence of contamination with adventitious agents was observed, due to the test article Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7.

#### I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent adventitious agent(s). The experimental design utilizes the inoculation of adult guinea pigs. The test is performed as described in CFR Title 21, Section 630.16(a)(4).

Adult guinea pigs are used in this assay to detect possible contamination of the test article with  $\underline{\mathbf{M}}$ .  $\underline{\mathbf{tuberculosis}}$  or other adventitious agent(s). Animals are examined for signs of illness, and any that become sick or show any abnormalities are examined in an attempt to determine the cause of illness or death.

#### II. STUDY INFORMATION

- A. Title: Animal Safety Test in Adult Guinea Pigs
- B. Study Number: ZA357.005102
- C. Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7 was received at Microbiological Associates, Inc. on 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.
- D. Medium Test Article: none
- E. Control Articles:
  - 1. Positive Control: none
  - 2. Negative Control: none
  - 3. Vehicle Control: none
- F. Test System: Guinea Pigs

Hartley Albino 6 adult females,

Body weight range: 350-400 g

Source: Hazleton Research Animals

Denver, Pennsylvania

G. Sponsor: Program Resources, Inc.

Biomedical Services Division 7655 Old Springhouse Road

McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department

Microbiological Associates, Inc.

Life Sciences Center 9900 Blackwell Road

Rockville, Maryland 20850

Animal Facility:

Microbiological Associates, Inc.

5221 River Road

Bethesda, Maryland 20816

#### I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate

Study Director: Janet Luczak, M.T. (ASCP), M.G.A.

#### J. Schedule:

Study Initiation Date: 07/11/90

2. Lab Initiation Date: 07/12/90

3. Lab Completion Date: 09/06/90

4. Study Completion Date: See Study Director's

Signature Date, in the

"Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

#### L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

#### III. PROCEDURES

A. Objective:

The study objective is to detect adventitious agent(s) that might be present in the test article.

#### B. Methods:

1. Test System Identification and Randomization

Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Animals were housed separately and were identified by ear tags.

The guinea pigs were randomized according to SOP OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. Three were held as uninoculated controls. All animals were observed every working day of the 42 day test period for death or clinical signs of illness or distress. Beginning day 21 post inoculation, rectal temperatures were recorded through day 42 post-inoculation. All remaining guinea pigs were sacrificed. Gross pathological examinations were performed on all guinea pigs.

#### 3. Animal Husbandry

- a. Animals were fed Ralston Purina Certified Guinea Pig Chow.
- b. Water was supplied <u>ad libitum</u> via water bottles. Water was disinfected with 7 ppm chlorine.
- c. Bedding Bed-o-Cobs, Anderson Cob Mills. Cages were changed as necessary, usually twice per week.
- d. Animal facilities are accredited by the American Association for Accreditation of Laboratory Animal Care.



TABLE 1

Group #	No. of Animals	Routes of Inoculation	Volume of Inoculum	Inoculum	Treatment after Inoculation
1	1.	I.C. I.P.	0.1 ml 5.0 ml	Test Article	Observe for Illness Record Rectal Temp. On Days 21-42 Post- Inoculation
2	1	SAA	SAA	SAA	SAA
3	1	SAA	SAA	SAA	SAA
4	1	None	None	Uninoc Control	SAA
5	1	SAA	SAA	SAA	SAA
6	1	SAA	SAA	SAA	SAA

SAA = Same As Above
I.C. = Intracranial
I.P. = Intraperitoneal

.

#### IV. RESULTS

All of the uninoculated control guinea pigs and all of the test article inoculated guinea pigs appeared normal and healthy throughout the 42 day observation period. See Table 2 for a summary of the data.

None of the uninoculated control guinea pigs and none of the test article inoculated guinea pigs had significant temperature rises indicative of either viral or bacterial infections during the 21 day recording period from day 21 through day 42 post-inoculation.

At examination, on day 42, for gross pathology, no lesions were found in the uninoculated control or test article guinea pigs. (See Pathology Report in Appendix.)

#### V. CONCLUSIONS

No evidence of contamination with adventitious agent(s) due to the test article, Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7, was observed.

#### VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman

Study Director

Date

TABLE 2

Summary of Daily Observations
for Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRbL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post- inoc.)	Day of Death/ Sacrifice (Post- inoc.	Range of Body Temp in <sup>O</sup> C D-21 to D-42
Guinea Pig	Test Article	10407 10408 10409	Normal Normal Normal		3	88.0 - 38.8 88.1 - 38.9 7.9 - 38.7
	Uninoc- ulated Control	10404 10405 10406	Normal Normal Normal		3	88.0 - 38.5 88.2 - 39.0 88.0 - 38.9

#### QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT GUINEA PIGS

Study Number: ZA357.005102

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/16/90 - 07/16/90, TO STUDY DIR 07/16/90, TO MGMT 07/16/90 PHASES: PROTOCOL REVIEW

INSPECT ON 08/23/90 - 08/23/90, TO STUDY DIR 08/23/90, TO MGMT 08/24/90 PHASES: EXAM. OF ABDOMINAL AND THORACIC VISCERA AT DAY 42 POST-

INOCULATION FOR OBVIOUS OR SUGGESTIVE ABNORMALITIES

INSPECT ON 09/12/90 - 09/12/90, TO STUDY DIR 09/12/90, TO MGMT 09/14/90 PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

granme Bowan	9/14/90
Joan M. McGowan	Date
Quality Assurance Unit	

VIII. APPENDIX

CORPORATE OFFICES
Life Sciences Center
9900 Blackwell Road • Rockville • Maryland 20850
(301) 738-1000 • Telex 908793 • Fax (301) 738-1036

GETHESDA LABORATORIES
5221 River Road • Bethesda • Maryland 20816
(301) 654-3400 • Telex 90-8793

#### PATHOLOGY REPORT

CAHS-2190		DATE RECEI	<b>VED:</b> 08/23/90
source:	Biotech Services ZA356.005102 ZA357.005 ZA358.005102 ZA359.005 ZA360.005102 ZA361.005	5102	•
SPECIES:	Guinea Pig Results of gross examina	ation according	to SOP #865.201.
	ZA356.005102		
	2190-1 (10401) Te Gross: No lesions for	est Article and	563.9 g
	2190-2 (10402) Te Gross: No lesions for	est Article and	684.2 g
	2190-3 (10403) Te Gross: No lesions for	est Article and	614.8 g
	2190-4 (10404) Co Gross: No lesions for	ontrol ind	614.4 g
	2190-5 (10405) Co Gross: No lesions for	ontrol ind	673.3 g
	2190-6 (10406) Co Gross: No lesions for	ontrol und	646.2 g
	ZA357.005102		
	2190-7 (10407) Te Gross: No lesions for	est Article and	629.0 g
	2190-8 (10408) Te Gross: No lesions for	est Article and	583.6 g
	2190-9 (10409) Te , Gross: No lesions for	est Article und	528.1 g

## PATHOLOGY REPORT

CAHS-2190

SOURCE: Biotech Services

PAGE 2

## ZA358.005102

2190-10 (10410) Test Article Gross: No lesions found	636.3 g
2190-11 (10411) Test Article Gross: Retroperitoneal abscess peritonitis	490.5 g (4x6 cm) and
2190-12 (10412) Test Article Gross: No lesions found	595.5 g
ZA359.005102	
2190-13 (10421) Test Article Gross: No lesions found	
2190-14 (10422) Test Article Gross: No lesions found	631.8 g
2190-15 (10423) Test Article Gross: No lesions found	547.4 g
2190-16 (10424) Control Gross: No lesions found	651.5 g
2190-17 (10425) Control Gross: No lesions found	623.6 g
2190-18 (10426) Control Gross: No lesions found	615.9 g
ZA360.0051)2	
2190-19 (10427) Test Article Gross: No lesions found	561.8 g
2190-20 (10428) Test Article Gross: No lesions found	594.6 g
2190-21 (10429) Test Article Gross: No lesions found	598.4 g

PATHOLOGY REPORT CAHS-2190 SOURCE: Biotech Services PAGE 3

#### ZA361.005102

2190-22 (10430) Gross: No lesions	Test found	Article	559.8 g
2190-23 (10431) Gross: No lesions	Test found	Article	547.9 g
2190-24 (10432) Gross: No lesions		Article	624.8 g

#### COMMENT:

The retroperitoneal abscess in guinea pig #2190-11 is believed to be due to a rectal perforation (no longer visible) caused by insertion of a temperature measuring probe. This is a common lesion in guinea pigs in which the probe is inserted on multiple occasions.

Anton M. Allen, DVM, Pin.D.

Director of Veterinary Services

# ANIMAL SAFETY TEST IN ADULT GUINEA PIGS

Study No.: ZA358.005102

Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7

Final Report

Program Resources, Inc. Biomedical Services Division 7655 Old Springhouse Road McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

## TABLE OF CONTENTS

		Page
	Summary	125
I.	Introduction	126
II.	Study Information	126
III.	Procedures	127
IV.	Results	130
v.	Conclusions	130
VI.	Approvals	130
VII.	Quality Assurance Statement	132*
ZIII.	Appendix	133

#### SUMMARY

The purpose of this test is to detect the presence of adventitious agent(s) which might be present, in the test article, preclarified bulk live virus and/or fluids, other than the specific virus in the product, by the inoculation and observation of guinea pigs.

No evidence of contamination with adventitious agents was observed, due to the test article Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7.

#### ZA358.005102

#### I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent adventitious agent(s). The experimental design utilizes the inoculation of adult guinea pigs. The test is performed as described in CFR Title 21, Section 630.16(a)(4).

Adult guinea pigs are used in this assay to detect possible contamination of the test article with M. tuberculosis or other adventitious agent(s). Animals are examined for signs of illness, and any that become sick or show any abnormalities are examined in an attempt to determine the cause of illness or death.

#### II. STUDY INFORMATION

- A. Title: Animal Safety Test in Adult Guinea Pigs
- B. Study Number: ZA358.005102
- C. Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7 was received at Microbiological Associates, Inc. on 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.
- D. Medium Test Article: none
- E. Control Articles:
  - 1. Positive Control: none
  - 2. Negative Control: none
  - 3. Vehicle Control: none
- F. Test System: Guinea Pigs
  Hartley Albin

Hartley Albino 6 adult females,

Body weight range: 350-400 g

Source: Hazleton Research Animals

Denver, Pennsylvania

G. / Sponsor: Program Resources, Inc.

Biomedical Services Division

7655 Old Springhouse Road

McLean, VA 22102

Authorized Representative: Dr. Louis Potash

#### ZA358.005102

H. Testing Facility: Biotechnology Services Department

Microbiological Associates, Inc.

Life Sciences Center 9900 Blackwell Road

Rockville, Maryland 20850

Animal Facility: Microbiological Associates, Inc.

5221 River Road

Bethesda, Maryland 20816

#### I. Personnel:

1. Study Director: Mary D. Whiteman

 Associate Study Director: Janet Luczak, M.T. (ASCP), M.G.A.

#### J. Schedule:

1. Study Initiation Date: 07/11/90

2. Lab Initiation Date: 07/12/90

3. Lab Completion Date: 09/06/90

4. Study Completion Date: See Study Director's

Signature Date, in the

"Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

#### L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

#### III. PROCEDURES

A. Objective:

The study objective is to detect adventitious agent(s) that might be present in the test article.

#### B. Methods:

1. Test System Identification and Randomization

Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Animals were housed separately and were identified by ear tags.

The guinea pigs were randomized according to SOP OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. Three were held as uninoculated controls. All animals were observed every working day of the 42 day test period for death or clinical signs of illness or distress. Beginning day 21 post inoculation, rectal temperatures were recorded through day 42 post-inoculation. All remaining guinea pigs were sacrificed. Gross pathological examinations were performed on all guinea pigs.

### 3. Animal Husbandry

- a. Animals were fed Ralston Purina Certified Guinea Pig Chow.
- b. Water was supplied <u>ad libitum</u> via water bottles. Water was disinfected with 7 ppm chlorine.
- c. Bedding Bed-o-Cobs, Anderson Cob Mills. Cages were changed as necessary, usually twice per week.
- d. Animal facilities are accredited by the American Association for Accreditation of Laboratory Animal Care.



## ZA358.005102

TABLE 1

Group #	No. of Animals	Routes of Inoculation	Volume of Inoculum	Inoculum	Treatment after <u>Inoculation</u>
1	1	I.C. I.P.	0.1 ml 5.0 ml	Test Article	Observe for Illness Record Rectal Temp. On Days 21-42 Post- Inoculation
2	1	SAA	SAA	SAA	SAA
3	1	SAA	SAA	SAA	SAA
4	1	None	None	Uninoc Control	SAA
5	1	SAA	SAA	SAA	SAA
6	1	SAA	SAA	SAA	SAA

SAA = Same As Above
I.C. = Intracranial
I.P. = Intraperitoneal

#### IV. RESULTS

All of the uninoculated control guinea pigs and two of the three test article inoculated guinea pigs appeared normal and healthy throughout the 42 day observation period. One of the test article inoculated guinea pigs (10411) was noted to appear lethargic with a rough hair coat and decreased fluid intake on day 35 post-inoculation. By day 38 post-inoculation, the animal appeared normal. See Table 2 for a summary of the data.

None of the uninoculated control guinea pigs and none of the test article inoculated guinea pigs had significant temperature rises indicative of either viral or bacterial infection during the 21 day recording period from day 21 through day 42. See Table 2 for a summary of the data.

At examination on day 42 for gross pathology, no lesions were found in any of the uninoculated control animals and no lesions were found in two of the three test article inoculated animals. One of the test article inoculated animals (10411) was found to have a (4 x 6 cm) retroperitoneal abscess and peritonitis. The abscess was attributed to a rectal perforation caused by insertion of a temperature measuring probe and was considered to be a common lesion in guinea pigs in which a probe is inserted on multiple occasions. (See Pathology Report in Appendix.)

#### V. CONCLUSIONS

No evidence of contamination with adventitious agent(s) due to the test article, Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7, was observed.

#### VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman

Study Director

ate

TABLE 2

Summary of Daily Observations
for Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRbL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post- inoc.)	Day of Death/ Sacrifice (Post- inoc.	Range of Body Temp in <sup>O</sup> C D-21 to D-42
Guinea Pig	Test Article	10410 10411 10412	Normal _a Normal	35		37.8 - 38.8 38.0 - 38.8 37.9 - 38.6
	Uninoc- ulated Control	10404 10405 10406	Normal Normal Normal		3	38.0 - 38.5 38.2 - 39.0 38.0 - 38.9

<sup>&</sup>lt;sup>a</sup> Animal had a rough hair coat and appeared lethargic with a decreased fluid intake day 35 post-inoculation. Animal appeared normal by day 38 post-inoculation

#### QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT GUINEA PIGS

Study Number: ZA358.005102

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Fractice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/16/90 - 07/16/90, TO STUDY DIR 07/16/90, TO MGMT 07/16/90 PHASES: PROTOCOL REVIEW

INSPECT ON 08/23/90 - 08/23/90, TO STUDY DIR 08/23/90, TO MGMT 08/23/90 PHASES: RECTAL TEMPERATURE DETERMINATION

INSPECT ON 09/12/90 - 09/12/90, TO STUDY DIR 09/12/90, TO MGMT 09/14/90 PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Joan M. McGowan Date
Quality Assurance Unit

VIII. APPENDIX

CORPORATE OFFICES
Life Sciences Center
9900 Blackwell Road • Rockville • Maryland 20850
(301) 738-1000 • Telex 908793 • Fax (301) 738-1036

BETHESDA LABORATORIES 5221 River Road • Bethesda • Maryland 20816 (301) 654-3400 • Telex 90-8793

### PATHOLOGY REPORT

CAHS-2190	·	DATE RECE	IVED:	08/23/90
SOURCE:	Biotech Services ZA356.005102 ZA357.005102			• •
	ZA358.005102 ZA359.005102 ZA360.005102 ZA361.005102	DATE REPO	RTED:	09/06/90
SPECIES:	Guinea Pig Results of gross examination	according	to SOP #	865.201.
	ZA356.005102			
	2190-1 (10401) Test And Gross: No lesions found	rticle	563.9 g	
	2190-2 (10402) Test And Gross: No lesions found	rticle	684.2 g	
	2190-3 (10403) Test And Gross: No lesions found	rticle	614.8 g	
	2190-4 (10404) Control Gross: No lesions found	L	614.4 g	
	2190-5 (10405) Control Gross: No lesions found	L	673.3 g	
	2190-6 (10406) Control Gross: No lesions found	L	646.2 g	
	ZA357.005102			
	2190-7 (10407) Test An Gross: No lesions found	rticle	629.0 g	
	2190-8 (10408) Test An Gross: No lesions found	rticle	583.6 g	
	2190-9 (10409) Test Ar , Gross: No lesions found		528.1 g	

# PATHOLOGY REPORT

CAHS-2190

SOURCE: Biotech Services

PAGE 2

# ZA358.005102

2190-10 (10410) Test Article Gross: No lesions found	636.3 g
2190-11 (10411) Test Article Gross: Retroperitoneal abscess (4) peritonitis	490.5 g k6 cm) and
2190-12 (10412) Test Article Gross: No lesions found	595.5 g
ZA359.005102	
2190-13 (10421) Test Article Gross: No lesions found	605.3 g
2190-14 (10422) Test Article Gross: No lesions found	631.8 g
2190-15 (10423) Test Article Gross: No lesions found	547.4 g
2190-16 (10424) Control Gross: No lesions found	651.5 g
2190-17 (10425) Control Gross: No lesions found	623.6 g
2190-18 (10426) Control Gross: No lesions found	615.9 g
ZA360.005102	
2190-19 (10427) Test Article Gross: No lesions found	561.8 g
2190-20 (10428) Test Article Gross: No lesions found	594.6 g
2190-21 (10429) Test Article Gross: No lesions found	598.4 g

PATHOLOGY REPORT CARS-2190

SOURCE: Biotech Services

PAGE 3

#### ZA361.005102

2190-22	(10430)	Test Article	559.8 g
			_

Gross: No lesions found

2190-23 (10431) Test Article 547.9 g

Gross: No lesions found

2190-24 (10432) Test Article 624.8 g

Gross: No lesions found

COMMENT: The retroperitoneal abscess in guinea pig #2190-11 is

believed to be due to a rectal perforation (no longer visible) caused by insertion of a temperature measuring probe. This is a common lesion in guinea pigs in which

the probe is inserted on multiple occasions.

Anton M. Allen, DVM, Ph.D.

Director of Veterinary Services



CORPORATE OFFICES
Life Sciences Center
9900 Blackwell Road • Rockville • Maryland 20850
(301) 738-1000 • Telex 908793 • Fax (301) 738-1036

BETHESDA LABORATORIES 5221 River Road • Bethesda • Maryland 20816 (301) 654-3400 • Telex 908793 Fax (301) 654-8916

December 17, 1990

Dr. Louis Potash Program Resources, Inc. Biomedical Services Division 7655 Old Springhouse Road McLean, VA 22101

Dear Dr. Potash,

Microbiological Associates, Inc. is an AAALAC accredited animal facility, and all studies are performed in accordance with the "Guide for the Care and Use of Laboratory Animals", U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, NIH Publication No. 86-23.

Sincerely,

Mary D. Whiteman

Study Director, In Vivo Assays

Biotechnology Division



# Program Resources, Inc.

Biomedical Services Division
7655 Old Springhouse Rd.
McLean, VA 22102 • (703) 506-0190
FAX (703) 506-0194

May 14, 1991

TO:

Mr. Donald Holzworth, Vice President Dr. Louis Potash, Study Director

FROM:

James R. Plautz Sr. QA Advisor

RE:

GLP Compliance Audit of Final Reports for Safety Testing of Dengue Virus Type 1 and Type 4

On April 14, 1991 a complete audit for GLP compliance (21 CFR, Part 58) was conducted for the subject final reports and their respective raw data.

Our complete findings indicate that the studies were conducted under the guidance of the referenced Standard Operating Procedures (SOPs), the variations from the SOPs had no apparent effect on study outcome, and that the final report for each study is substantiated by the raw data.

Animal safety testing was conducted and reported separately from these final reports.

Janes R. Vlaut May 14, 1999

# APPENDIX II

Dengue-4 Virus Strain Carib 341750

#### **FOREWORD**

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

Where coyrighted material is quoted, permission has been obtained to use such material.

Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or serices of these organizations.

In conducting research using animals, the investigator(3) adhered to the "Guide for the Care and Use of Laboratory Animals", prepared by the committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIS Publication No. 35-23, Revised 1985) - (see Attachment A).

For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45 CFP 46.

PI Signature

Date

# TABLE OF CONTENTS

	Forewo	ord	••••••	1
I.	Intro	duct	ion	4
II.	Synops	sis		4
III.	Differ	cent	Summary Relating to the Safety Testing of Three (3) Passage Levels of Dengue Virus Type 4 (Carib 341750) on Seeds: Propagated in DBS-FRhL-2 Cell Cultures	6
	A.	I:ıc	cula	6
	В.		ety Testing Procedures and Results on the Crude vest Fluids	6
		1.	Microbial Sterility	6
		2.	Identity in Tissue Culture (Serum-neutralization)	7
		3.	Purity (Safety) in Tissue Cultures	7
		4.	Animal Safety	9
Table	s: I a	nd 1	II	10
Appen	dixes:	A	***************************************	16
		В	•••••	20
		С	,	54
		D	•••••	100
Attac	hment:	A	•••••	143
Attac	:hment:	В	Quality Assurance Statement from Program Resources, Inc.	144

#### I. INTRODUCTION

The accompanying protocol is a description of the safety testing of 3 crude harvest lots of dengue virus type 4 designated as:

Dengue Virus Type 4 (Carib 341750): PDK-6, FRhL-2/d7: PDK-10, FRhL-2/d7 and PDK-15, FRhL-2/d7 of 9 March 1990

Utilizing the testing procedures herein described, this fluid is considered to have not passed satisfactorily all tests for safety including purity. The detailed records with respect to passage history, pool production, and subsequent safety testing may be found in the laboratory notebooks located at:

The Walter Reed Army Institute of Research (WRAIR), Eldg. 501, Washington, DC 20307-5100 - (Dr. Ken Eckels)

The Experimental Virus Vaccine Production Laboratory - Suite #500 - (Flow Laboratories, Inc.) Program Resources, Inc. [PRI], McLean, VA - (Dr. Louis Potash)

All procedures performed at PRI followed Gccd Laboratory Practices (GLP) regulations (21 CFR, Part 58) and were carried out in accordance with the guidelines established by the FDA for live and inactivated vaccines as found in 21 CFR, Parts 610.11, 610.12, 610.30, 630.10 - 630.17, etc. of April 1989. These procedures are detailed in the following SOPs and recorded on the indicated VVPL Forms:

```
SCP No.: 500.001 - Issued 29 Oct 1980, Revised 13 Feb 1986
                                            18 Feb 1986
        500.002 - "
                        29 Oct 1980, "
        500.008 -
                        13 Jan 1901,
                                             3 Mar 1986
 VVPI FORM #008 - Issued 29 Cct 1980, Revised 3 May 1984
                        15 Jan 1981, "
                                            13 July 1984
           016 -
                    Ħ
           017 -
                        16 Jan 1981,
                                            13 Jan 1986
           019 -
                        8 Oct 1984
```

#### II. SYNOPSIS

A. Crude Virus Harvests:

Dengue Virus Type 4 (Carib 341750) PDK-6, FRhL-2/d7 of 9 Mar 90 PDK-10, FRhL-2/d7 of 9 Mar 90 PDK-15, FRhL-2/d7 of 9 Mar 90

- B. Safety Tests on Crude Harvest Fluids:
  - Sterility: Fluid Thioglycollate (FTM), Tryptone Sova Broth (TSB), Mycoplasma

pcciic co/a baccii (205)	,	
PDK- 6 Virus Fluid	(47 ml)	No Growth
PDK-10 Virus Fluid	(47 ml)	No Growth
PDK-15 Virus Fluid	(47 ml)	No Growth
Control Fluid (TCF)	(47 ml)	No Growth
	PDK- 6 Virus Fluid PDK-10 Virus Fluid PDK-15 Virus Fluid	PDK-10 Virus Fluid (47 ml) PDK-15 Virus Fluid (47 ml)

 Tissue Culture Identity and Purity (Safety): AGMK, PHA, FRhL-2, PRK, and Flow 5000.

	ppu 6 111 p7 14	/OC 1 \	Manking Commonst
a.	PDK- 6 Virus Fluid	(25 ml)	Unsatisfactory*
b.	PDK-10 Virus Fluid	(25 ml)	Unsatisfactory*
c.	PDK-15 Virus Fluid	(25 ml)	Unsatisfactory*
	Control Fluid (TCF)	(25 ml)	Satisfactory

- 3. Animal Safety:
  - a. Pabbits: I.D. & S.Q. (Appendix B)

    (1) PDK- 6 Virus Fluid (30 ml) Satisfactory

    (2) PDK-10 Virus Fluid (30 ml) Satisfactory

    (3) PDK-15 Virus Fluid (30 ml) Satisfactory
  - b. Adult Mice: I.C. & I.P (Appendix C)
    (1) PDK -6 Virus Fluid (10.6 ml) Satisfactory
    (2) PDK-10 Virus Fluid (10.6 ml) Satisfactory
    (3) PDK-15 Virus Fluid (10.6 ml) Satisfactory

<sup>\*</sup> Test unsatisfactory only in the AGMK test system. Non-descript morphological changes observed in primary AGMK flask cultures, particularly after films were stained. All AGMK tube subcultures exhibited varying degrees of cytopathology ranging from 1-3+. Both flask and tube subcultures were negative for hemadsorption. All tube subcultures completely inhibited the Coxsackie A-9 challenge virus.

# 3. Animal Safety (continued):

- c. Suckling Mice: I.C. & I.P. (Appendix C)

  (1) PDK-6 Virus Fluid\* (2.2 ml) Satisfactory

  (2) PDK-10 Virus Fluid\* (2.2 ml) Inconclusive\*\*

  (3) PDK-15 Virus Fluid\* (2.2 ml) Inconclusive\*\*\*
- d. Guinea Figs: I.C. & I.P. (Appendix D)
  - (1) PDK -6 Virus Fluid (15.3 ml) Satisfactory (2) PDK-10 Virus Fluid (15.3 ml) Satisfactory (3) PDK-15 Virus Fluid (15.3 ml) Satisfactory
- \* Virus fluid was mixed with equal parts of a 1:5 dilution of the immune serum and incubated at 37°C for 90 minutes prior to inoculation.
- \*\* Although all of the 20 sucklings appeared normal and survived the initial 14-day incubation period, only 9 of the 20 sucklings incculated with their emulsified tissue survived the final 14-day blind passage with 2 lethargic and with hunched postures.
- \*\*\* Cnly 6 of 15 inoculated sucklings appeared normal at the end of the initial 14-day incubation period and were emulsified for blind passage. Of the other 10 original sucklings, 1 was apparently cannibalized, 4 were found dead (marked autolysis on necrosy & histopathology) and 5 were found moribund and were homogenized for subpassage. Hone of the 20 sucklings inoculated with the 'normal' homogenate survived the final 14-day blind passage and, of the 20 sucklings inoculated with the moribund homogenate, 10 survived but of these 2 were moribund and 1 was lethargic and runted.

DETAILED SUMMARY RELATING TO THE SAFETY TESTING OF THREE (3) III. DIFFERENT PASSAGE LEVELS OF DENGUE VIRUS TYPE 4 (CARIB 341750) PRODUCTION SEEDS: PROPAGATED IN DBS-FRbL-2 CELL CULTURES

#### Α. Inocula

In May 1990, the following frozen materials were obtained for testing from Dr. K. Eckels, Contracting Officer's Representative, Walter Reed Army Institute of Research (WRAIR), Bldg. 501, Washington, D.C.:

- 1. Dengue-4 (Carib 341750) crude, unclarified harvest fluids of 9 Mar 90:
  - PDK- 6, FRhL2-2 (day 7 harvest)...... 20 x 10 ml vials a.
  - b.
  - PDK-10, FRhL2-2 (day 7 harvest)...... 20 x 10 ml vials PDK-15, FRhL2-2 (day 7 harvest)...... 20 x 10 ml vials
  - Control Fluids ...... 4 x 25 ml vials
- 2. Dengue-4 Antiserum: 814669 CAREC, SM-5 OF 11/21/82 ... 1 x 8 ml

On arrival in this laboratory, the virus and control fluids were stored at  $-70^{\circ}$ C, or below, and the antiserum at  $-20^{\circ}$ C, or below.

#### Safety Testing Procedures and Results on the Crude, Unclarified B. Harvest Fluids (SOP No.: 500.008)

# Microbial Sterility - (VVPL FORM #011)

Aliquots of the bulk frozen fluids were thawed and tested for microbial sterility as follows:

- Fluid Thioglycollate Medium FTM (LOT VVPL #030): Each of 10 culture tubes (9-10 ml medium per tube) was inoculated with 1 ml volumes of the crude virus fluids and each of 10 culture tubes was inoculated with 1 ml volumes of the crude control fluid. An additional 10 culture tubes were included as uninoculated controls. All cultures were vortex mixed and incubated at  $32^{\circ}C$  ( $\pm$   $2^{\circ}C$ ) for 21 days with periodic examination for evidence of growth. No growth was observed in any of the 50 culture tubes.
- Tryptone Soya Broth TSB (LOT VVPL #030): Each of 10 culture tubes (9-10 ml medium per tube) was inoculated with 1 ml volumes of the crude virus fluids and each of 10 culture tubes was inoculated with 1 ml volumes of the crude control fluid. An additional 10 cultures were included as uninoculated controls. All cultures were vortex mixed and incubated at 22  $^{\circ}$ C ( $^{+}$ 2  $^{\circ}$ C) for 21 days with periodic examination for evidence of growth. No growth was observed in any of the 50 culture tubes.

The results of the above described Microbial Sterility Assays are summarized in Table I.

c. Mycoplasma Sterility: These assays were performed by PRI's Mycoplasma Testing Laboratory and included both the routine PFLO agar and proth assays and the specific test for the detection of  $\underline{M}$ . hyporhinis. Samples (1 x 2 ml and 1 x 25 ml) of the 3 crude virus fluids and of the 1 control fluid were submitted for testing. All samples were reported to be negative for mycoplasmas. A copy of this report is appended to this Protocol - (Appendix A - 1, 2, 3 & 4).

# 2. Identity in Tissue Culture (Serum-Neutralization) -

 $\,$  Mo attempt was made to identity the crude virus pools in tissue cultures.

# 3. Purity (Safety) in Tissue Cultures - (VVPL FORM #016)

a. <u>Tissue Cultures</u>: All flask and roller tube cell cultures were prepared by contract personnel. Cultures were maintained on Medium MEM containing 5 to 10% fetal bovine serum (heat-inactivated) plus antipictics: gentamicin @ 100 mcg/ml; necmycin @ 50 mcg/ml; and amphotericin B (I.V.) @ 2.5 mcg/ml. Cultures were incculated, refed and subpassaged as indicated below. The following tissue culture systems were utilized:

(1)	Tertiary African Green Monkey Kidney (AGMK)	MEM	+	5% :	serum
(2)	Primary Human Amnion (PHA)	MEY	+	10% \$	serum
(3)	Fetal Rhesus Lung (FRhL-2)	MEM	+	5% গ	serum
(4)	Primary Rabbit Kidney (PRK)	MEM	÷	5% \$	serum
	Whole Human Embryo Fibroblast (Flow 5000)				

# b. General Testing Procedures

### (1) Crude Virus Fluids

(a) Primary Flask Cultures: Equal 5 ml volumes of the bulk crude virus fluids and of a 1:10 dilution of the rabbit immune serum (Den-4, CAREC 814669, smb 5) were well mixed and incubated at 37°C (water bath) for 90 minutes. Due to the small volume of antiserum available, only 5 ml of each she virus fluids were tested per tissue culture system wherein 1 x 75 cm² flask per tissue culture system was inoculated with 10 ml of these scrum-virus mixtures. Plasks contained approximately 25 ml of maintenance medium at the time of inoculation. Cultures were incubated at 35°C (37°C for PHA) for 14 days with periodic microscopic examination for any signs of CPE and/or cellular degradation. When necessary to maintain the integrity of the cell films, cultures were refed with 35 ml of fresh medium.

(b) <u>Secondary Tube Subcultures</u>: On the 14th day of incubation, the primary cultures were re-examined microscopically and the fluids harvested individually and treated with the specific immune serum - 0.1 ml per harvest. In addition, to each individual harvest was added: 0.1 ml gentamicin (50 mg/ml); 1 ml penicillin-streptomycin solution (5000 units/ml and 5000 mcg/ml, respectively); and 10% of 10X SPG\* (v/v).

<sup>\* 10</sup>X SPG: sucrose, 2.18 M; KH<sub>2</sub>PO<sub>4</sub>, 0.038 M; K<sub>2</sub>HPO<sub>4</sub>, 0.072 M; monosculum glutamate, 0.049 M.

Following mixing, the fluids were incubated at room temperature for 60 min. and then subpassed into homologous roller tube cultures – 0.5 ml of each harvest into each of 20 tubes. The remainder of the harvest fluids was saved and stored at  $-75^{\circ}$ C, or below. All primary cultures were tested for hemadsorption by the addition of 0.1% guinea pig RBC (in PBS) and incubation at 4°C for a minimum of 30 minutes. All cultures were negative for hemadsorption.

Tube cultures (refed with 2 ml of maintenance medium prior to inoculation) were incubated at 35°C (37°C for PHA) for 13-14 additional days. When necessary to maintain the integrity of the cell films, cultures were refed with 2 ml of fresh medium. Cultures were examined microscopically at periodic intervals and at the end of the incubation period for any signs of CPE. After final examination, tubes were divided - depending on the specific cell system - for additional testing:

AGMK, PHA, FRhL-2 and Flow 5000 Tube Cultures: These were divided into 3 groups as follows:

1/4th tested for the presence of hemadsorbing agents,

1/4th fixed and stained with a solution of 5% glutaraldehyde + 1:10 giemsa stain and examined microscopically for any CPE,

1/2 Challenged with Coxsackie A-9 virus (0.2 ml per tube at dilutions noted in the tables) for the detection of non-CPE producing agents and/or latent agents via the interference phenomenon.

PRK Tube Cultures: These were equally divided into 2 groups:

1/2 tested for the presence of hemadsorbing agents,

1/2 fixed and stained with the glutaraldehyde-giemsa stain solution and examined microscopically for any CPE.

No challenge studies were carried out with the Coxsackie  $\Lambda$ -9 virus since this virus does not produce any discernible CPE in this tissue culture system.

# (2) Crude Control Fluid

A single 75 cm<sup>2</sup> flask per tissue culture system was incculated with 10 ml of crude control fluid. Cultures were handled in a manner similar to that described above for the crude virus fluid except that immune serum was not included.

# (3) Uninoculated Cell Lot Controls

Two  $\times$  75 cm<sup>2</sup> flasks per tissue culture system were included as uninoculated cell lot controls and were handled in a manner similar to that described above for the crude virus fluid except that immune serum, was not included. In addition, an appropriate number of uninoculated roller tube cultures were included as cell lot controls for the secondary tube subcultures.

In all challenge studies, l to 4 culture tubes per set were left unchallenged to serve as controls to the challenge virus.

The results of these in vitro Tissue Culture Purity (Safety) tests are summarized in Tables II-A through -E.

# 4. Animal Safety Tests

Due to the dismantling of Flow's Animal Facility during December 1989, all animal studies were performed by Microbiological Associates, Inc. The inocula for these animal studies were the three crude virus suspensions

- a. Adult Rabbits MBA Studies #ZA359.005101, #ZA360.005101 and #ZA361.005101 these tests were reported to be satisfactory and copies of these Final Reports may be found in Appendix B.
- b. Adult and Suckling Mice MEA Studies #2A359.005100, #2A360.005100 and #2A361.005100 all three tests in adult mice were reported to be satisfactory. However, of the three sucking mice tests, only Study #2A359.005100 (PDK-6, FRhL-2/d7 inoculum) was reported to be satisfactory, whereas the tests in the other 2 studies (#2A360.005100 and #2A361.005100 PDK-10 & PDK-15) were reported to be inconclusive due to the lethal effect of the test articles. Copies of these Final Reports may be found in Appendix C.
- c. Adult Guinea Pigs MBA Studies #ZA359.005102, #ZA360.005102 and #ZA361.005102 these tests were reported to be satisfactory and copies of these Final Roports may be found in Appendix D.

Alicrobial Sterility Test Results on the Crude Dengue-4 Virus (Carib 341750) Production Seed Pools Table I.

		Vol. per culture	Marine Co-Company of Contract	Da	Date	
Culture Medium	No.	(m1)	Temperature	On Test	Off Test	Results
1.						
Fluid Thioglycollate						
(FIM) LOT VVPL-#030	10		35°C ( <u>+</u> 2°C)	11/12/90	12/03/90	No Growth
PDK- 6 Virus Fluid	10	1.0		_		No Growth
PDK-10 Virus Fluid	10	1.0			_	No Growth
PDK-15 Virus Fluid	1.0	1.0		_	_	No Growth
Control Fluid	10	1.0		11/12/90	12/03/90	No Growth
Tryptone Soya Broth						
(TSB) LOF VVPL #030	10		55°C (±2°C)	11/12/90	12/03/90	No Growth
PDK- 6 Virus fluid	10	1.0		<u></u>		No Growth
PDK-10 Virus Fluid	10	1.0				No Growth
PDK-15 Virus Fluid	10	1.0		_		No Growth
Control Fluid	10	1.0		11/12/90	12/03/90	No Growth
		<b>&gt;</b>		06/27/77		75/07/21

Tissue Culture Purity (Safety) Test Results on the Crude Dengue-4 Virus (Carib 341750) Production Seed Pools Table II.

A. Tertiary African Green Monkey Kidney (AGAK)

۶.						0.5 ml pe	per tube			
•	- 1	Initial Flasks	asks		<u>ቤ</u>	Passage #J				
	Lot #16.	18 (#2129)	9, p4))	Lot # 1656	56 (2129	, p3)				
	Day 14			Day 14	14 + 14 = 28	8				
							Coxs	ackie A-	Coxsackie A-9 Challenge*	enge*
Material Tested	CPE	Hads	Stain	CPE	Hads	Stain	10-3	10-4	10_5	10_6
			**			***				
PDK- 6 Virus/Serum Mixture	0/1	0/1	1/1	20/20	0/5	5/2	0/3	0/2	0/2	0/2
) Virus/Serum Hixture	0/1	0/1	1/1	20/20	0/ 5	5/5	0/2	0/2	0/2	0/5
Virus/Serum Mixture	77	1/0	1/1	20/20	0/5	5/5	0/2	0/2	0/2	0/2
ol Fluid (TCF)	1/0	0/1	0/1	0/20	0/ 5	0/ 5	2/2	2/2	1/2	0/2
Control - (1) Control - (2)	0/2	0/2	0/2	0/40	0/10 0/12	0/10 0/12	4/4 8/8	4/4	4/4	2/4

Coxsackic A-9 Challenge Results based on a 6-day incubation at 35°C. Prior to challenge, all tubes refed with 2 ml of fresh medium Complete inhibition of Coxsackie A-9 challenge virus by virus/serum mixture series. \*

Stained flasks revealed non-descript CPE in virus/serum inoculated flasks only. \*

cytopathology on day 20 (days 14 + 6) which was attributed to dengue virus breakthroughs. All tubes inoculated with harvests from virus/serum inoculated flasks exhibited

Staining of tubes confirmed the varying degrees of cytopathology: PDK-6 = PDK-15 < PDK-10. \*\*\*

Tissuc Culture Purity (Safety) Test Results on the Crude Dengue-4 Virus (Carib 341750) Production Seed Pools Table II.

B. Primary Human Annion (PHA)

			suge*	10-6	1/2	0/5	0/2	0/5	2/4
		-	Coxsackie A-9 Challenge*	10-5	2/2	1/2	0/2	2/2	4/4
			ckie A-	10-4	2/2	2/2	2/2	2/2	4/48/8
er tube a #1			Coxsa	10-3	2/2	2/2	2/2	2/2	4/4
0.5 ml per tube Passade #1		23		Stain	0/ 5	0/5	0/5	0/ 2	0/10 0/12
	639	Day: $14 + 14 = 23$		Ilads	0/5	0/ 5	0/ 5	0/ 5	0/10 0/12
	Lot # 1639	Day: 14		CPE	0/20	0/20	0/20	0/20	0/40
				Stain	N O N	ON ON	QN	S	DE
Inicial Flasks	1541	p.		Hads	0/1	0/1	1/0	0/1	0/2
Inicial	Lot # 1	Day: 1	1	CPF	1/0	77	0/1	0/1	0/2
	•		•	Material Tested	DK- 6 Virus/Serum Nixture	OK-10 Virus/Serum Mixture	DK-15 Virus/Serum Nixture	Control Fluid (TCF)	Control - (1) Control - (2)

- 12 -

ND = Not done

Coxsackie A-9 Challengs Results based on a 4-day incubation at 37°C. Prior to challenge, all tubes refed with 2 ml of fresh medium.

<sup>\*\*</sup> On day 7, all flasks were refed with 35 ml of fresh madium.

Tissue Culture Purity (Safety) Test Results on the Crude Dengue-4 Virus (Carib 341750) Production Seed Pools Table II.

C. Fetal Rhesus Lung (FRhL-2)

				*	ر ا	Ġ	~	2	2	21. W
				enge	10-5	6	7	6	1/2	3/4
				9 Chall	10-4	2/2	2/2	2/2	2/2	4/4
				Coxsackie A-9 Challenge*	10-3	2/2	2/2	2/2	2/2	4/4
r tube	<b>=</b>			Coxse	10-2	2/2	2/2	2/2	2/2	4/4
0.5 ml par tube	Passage #1		28		Stain	0/5	o/ 5	0/5	0/ 5	0/10 0/12
		687 p24	Day 14 + 14 = 28		Hads	5 /0	0/5	0/5	0/5	0/10 0/12
		Lot # 1687	Day 14		CPE	0/20	0/20	0/20	0/20	0/40
	sks				Stain	QN	QQ QQ	2	QN	MD
	Initial Flasks	1610 p2	Ü		Hads	0/1	1/0	77	0/1	0/2
		Lot # 1610	7.0		CPE	1/0	7/	0/1	0/1	0/2
	٠	- 1	. !	•	Material Tested	PDK- 6 Virus/Serum Mixture	10 Virus/Serum Mixture	.5 Virus/Serum Mixture	ntrol Fluid (TCF)	Control – (1) Control – (2)
						POK-	PDK-1	PDK-1	Contr	88

- 13 -

IID = Not done

Coxsackie A-9 Challenge Results based on a 3-day incubation at 35°C. Prior to challenge, all tubes refed with 2 ml of fresh medium.

Tissue Culture Purity (Safety) Test Results on the Crude Dengue-4 Virus (Carib 341750) Production Seed Fools Table il.

D. Primary Rabbit Kidney (PRK)

	Initial	l Plasks		0.5 n	0.5 ml per tube Passage #1	90
	Lot # 1650	90		Lot # 1693	593	
	Day: 14			Day: I	Day: 14 + 14 = 28	28
Material Tested	CPB	Hads	Stain	CPE	Hads	Stain
PDK -6 Virus/Serum Mixture	0/1,*	1/0	NO	0/20	0/10	0/10
K-10 Virus/Serum Mixcure	0/1*	ړ/	N ON	0/20	0/10	0/10
K-15 Virus/Serum Mixture	5	77	Q2	0/20	0/10	0/10
	0/1	0/1	Q.	0/20	0/10	01/0
Control - (1) Control - (2)	0/2	0/2	ΔN	0/40 0/24	0/20 0/12	0/20 0/12

Commencing on day 10, focal area of vacuolation observed in these flasks only. Whatever caused these morphological channes was not subpassaged into the tube subcultures.

ND = Not done

Tissue Culture Purity (Safety) Test Results on the Crude Dengue-4 Virus (Carib 341750) Production Seed Pools Table II.

E. Whole Numan Embryo Fibroblasts (Flow 5000)

				Coxsackie A-9 Challenge*	10-4 10-5				1/2 1/2	4/4 1/4
		بعدية ويوه ويوسه وخدود فيستفيدان		ckie A-9	10-3	2/2	2/2	2/2	2/2	4/4
r tube	~;			Coxsa	10-2	2/2	2/2	2/2	2/2	4/4
0.5 ml per tube	Passage #1		28		Stain	0/5	o   	o S	0/ 5	0/10
		669 120	+ 34 = 5		Hacls	5 /0	o / s	ر در	ა გ	0/10
		Lot # 1669	Day 14		CPE	0/20	0/20	0/20	0/20	0/40
	isks	~			Stain	2	Ş	Ş	Ş	ð
	itial Fla	1630 ple			Hads	0/1	2	<u>ر</u>	2	0/2
	In	Lot # ]	3		CPE	1/0	2	ζ	2	0/2
	,	. '	. 1	•	Material Tested	PDK- 6 Virus/Sorum Mixturo	Virus/Serum Mixturo	Virus/Serum Mixture	Fluid (TCF)	Control - (1) Control - (2)
						POK- 6	FCK-10	PUK-15	Control	Cont

Coxsackie A-9 Challenge Results based on a 5-day incubation at  $35^{\rm O}{\rm C}$ . Prior to challenge, all tubes refed with 2 ml of fresh medium.

ND = Not done

# PRI

Program Resources, Inc. **Biomedical Services Division** 7655 Old Springhouse Rd.

McLean, VA 22102 • (703) 506-0190

FAX (703) 506-0194

8 August, 1990.

To: Dr. Louis Potash.

From: Jim Quartey. 50

Subject: Mycoplasma Testing. (Charge # 807)

This letter is to inform you that, the eight (8) samples listed below which you had submitted for the detection of Mycoplasma hyorhinis using the direct immunofluorescence staining and for the detection of

Mycoplasma in general using the DNA Hoechst stain and Agar testing were found to be negative.

a. Dengue-1 (#45AZ5) Production Seed of 16 Feb 90:

- 1. PDK-10, FRhL-2/\(\text{D7}\).
  2. PDK-20, FRhL-2/\(\text{d7}\).
- 3. PDK-27, FRhL-2/d7.
- 4. Control Fluid.

b. Dengue-4 (#341750) Production Seed of 9 Mar 90:

- 1. PDK-6, FRhL-2/d7
- 2. PDK-10, FRhL-2/d7
- 3.PDK -15,FRhL-2/d7
- 4. Control Fluid.

	Cate: 7/9/90 Se	et up by: 	1/21/90					Page 1 of /							
	Projected Final Read	iing:	/3/	// 2										e s	
	Billing Date:	,	811.7	ing //C	Mimp)				. 7 . 141	/3/	_		৪×	rample	١.
			Pre		inary		Hoed			al	L	Sub		] ]	
	Identification Number	7/	1	2	1	2	1	2	1		day		2	Notes	
	Negative Control		$\bigcirc$		0		0		0						
	Positive Control		+		+1		+		+		<u> </u>	ļ			
GUE	-1 (#45AZ5) , FRAL- 2/27	197	0	-	0		0		0						
(-)(	D. FRAL - 2/27	198	0	-	0		0		0			1	1		
v-0	7. FRAL - 2/d17	199	0		0		0		0						
	DL FZUID	200	0		0		0		0	<u> </u>					
,,,,,	OL PLOID	200				<u> </u>	<u> </u>				一		<del>                                     </del>		
							-				<del>                                     </del>	<del>                                     </del>	-		
GUE	=-4 (#34/750)	001					-					-	-		
	-4 (#341750) FRAL -2/d7				0	<u> </u>	0	<u> </u>	0	<u> </u>	_	-	-		
ベーノ	0, FRh1 - 2/d7	202	0		0		0		0		<u> </u>	<u> </u>	<u> </u>	<u> </u>	
K-1.	15, FRBL- 2/27	203	0		0		0		0		_	1	<u> </u>	<u> </u>	
コヘノブ	ROL FLUID	204	0		0		0		0					<u> </u>	
												1	1		
			F			İ							T		
		<u> </u>				<u> </u>	T	<b>†</b>		T	T		1	1	
		<u> </u>		<u> </u>	1		T	<u> </u>			1	<del>                                     </del>	†	1	<del></del>
		-		-	$I^{-}$	+	$\vdash$	+-	<del>                                     </del>	+	<b>†</b>	+	+	1	
		-	<del> </del>	t	t	$\vdash$	+	+-	$\vdash$	+	T	+-	$\dagger$	<del> </del>	
		<del> </del>	<del> </del>	-	$\vdash$	+-	+	+-	$\vdash$	<del>                                     </del>	+	+-	+-	+	
	Read by:	xxxxxxx	<b>K</b>	-	<b>k</b>	-	+	+	13>	+	+-	+	+	+	
	Date:	xxxxxxx	1	Ţ`	1	<u> </u>	13/	<del>9</del>	1 .	1	+	+-	+	<del> </del>	
		xxxxxx		1	125	1_	100	1_	7/34	1_	丄	<u> </u>			/

# MYCOPLASMA TEST RECORD SHEET

Date	1 IOT # Nerobic   Anaerobic   On Test   Off Test   Results   $\#34/750$ Virus Fluid - IOT # PDK-6 FRbL - $2/d7$ . Myc# 201	1 - 2 - 1 - 16/01 - 1 - 1 - 1	25.0 125.0 1/1/01	125. C 125. C 175. C 17/1/6, 17/2/6, NEGATIVE	1 . 2   . 2  ////   NEGATIVE	125:0 125.0 17/9/0/18/3/0/ NEGATIVE 1	Z	125	1 . 2 1 /2/701/ // NEGATIVE	#34/750) controlling - LOT # POK-10, FR16- 2/07, MYC# 20R	-2-17/9/6, 17/21/6,L	125.0 125 0 1/1/0 1/4/70   NEGATIVE	125.0125.017/1, 6,17/21/6,   NEGATIVE		17/1960 18/3/60 L	긻	1250125.017/46,18/8/9, L	1 2 3/10/10/10/
	Culture Medium   LOT #	ł I	PPLO Broth   900503	D 5 Subpass to Broth	to Agar	D10 Subpass to Broth	to Agar	D15 Subpass to Broth	to Agar	The	PPLO Agar	PPLO Broth	D 5 Subpass to Broth	to Agar	D10 Subpass to Broth	to Agar	D15 Subpass to Broth	to Agar

Negative Control (-): FB 29/0/ CO70 Positive Control (+): M. Olymini

Date: 8/8/90

Signed: Will Cox

- 18 -

# MYCOPLASMA TEST RECORD SHEET

	_	No. ml Tested	Rested	Date	te	
Culture Medium	LOT	Aerobic	Anaerobic	On Test	Off Test	Results
DENGUE-4/#	341750	Virus Fluid -	- 10T # PDA	1-15, FR	12-2/27	(#341750) Virus Fluid - LOT # POK-15, FRBL- 2/27. Myc#203
PPLO 'Agar	1900523	31 .2	.2	.2 17/9/.	17/01/2	NEGATIVE
PPLO Broth	1900503   25.0	125.0	25.0	11/20	01/2	NEGATIVE
D 5 Subpass to Broth		125.0	25.0	17/11/6,17/	17/3/601	NEGATIVE
to Agar		.2	. 2	110/70	1-1/70	NEGATIVE
D10 Subpass to Broth		125.0	125.0	17/10/6.	10/8/18/01	NEGATIVE
to Agar		2.	. 2	111/70	06/6/	NEGATIVE
D15 Subpass to Broth		125.0	25.0	1/2/1/	18/8/	NEGATIVE
to Agar		.2	3	141110	\	NEGATIVE
DENGUE-4/	752/HS#	#3#/750\control Fluid -	1 - LOT # //	Nc# 204.	o⊬.	
PPLO Agar		.2	Ġ	766	1/01/	NEGATIVE
PPLO Broth		125.01	25.0	11170	144/80	NEGATIVĚ
1 5 Subpass to Broth		125.0	25.0	11/1/6	1/2,6,1	NEGATIVE
to Agar	*	.2	.2	02/01/	0//2/	NEGATIVE
Dlt ibpass to Broth		25.0	25.0	7/10/6	18/3/6, 1	NEGATIVE
to Agar		.2	.2	11/10	17701	NEGATIVE
D15 Subpass to Broth		25.0	25.0	1/24/	18/6/51	NEGATIVE
to Agar	÷	. 2	S	0///	10/10	NEGATIVE

Negative Control (-): FB 2910/0070 Positive Control (+): M. asglmuni

Signed:

Date: 8/

# APPENDIX

В

# ANIMAL SAFETY TEST IN ADULT RABBITS

Study NO.: ZA359.0051	01						
Dengue-4 Prod Seed: P	PDK- 6,	FRhL-2/d7	• • • • • • • •	pages	21	-	31
Study NO.: ZA360.0051	01						
Dengue-4 Prod Seed: P	PDK-10,	FRhL-2/d7	•••••	pages	32	-	42
Study NO.: ZA361.0051	01						
Dengue-4 Prod Seed: P	PDK-15.	FRhL-2/d7		pages	43	_	53

#### ANIMAL SAFETY TEST IN ADULT RABBITS

Study No.: ZA359.005101

Test Article: Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7

Final Report For

Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

By Microbiological Associates, Inc. Life Sciences Center 9000 Blackwell Road Rockville, Maryland 20850

# TABLE OF CONTENTS

	•	Page
	Summary	23
I.	Introduction	24
II.	Study Information	24
III.	Procedures	25
IV.	Results	26
v.	Conclusions	26
VI.	Approvals	27
VII.	Quality Assurance Statement	31

#### SUMMARY

The purpose of this assay is to detect the presence of adventitious agent(s) in the test article pre-clarified bulk live virus vaccine and/or fluids, other than the specific virus in the product. The test article was inoculated into adult rabbits.

No evidence of contamination with adventitious agent(s) was observed due to the test article Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7.

#### I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent adventitious agent(s). The experimental design utilized inoculation of adult rabbits. The test is performed as described in CFR Title 21, Section 630.16.

Adult rabbits are utilized in this assay to detect possible contamination of the test article with B-virus or other adventitious agent(s) including other Simian agents, adenovirus(es), etc. which might be present in the test article. All animals are observed for signs of illness and any that become sick or show any abnormalities are examined in an attempt to establish cause of illness or death.

#### II. STUDY INFORMATION

- A. Title: Animal Safety Test in Adult Rabbits
- B. Study Number: ZA359.005101
- C. Test Article: Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7 was received at Microbiological Associates, Inc. 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.
- D. Medium Test Article: none
- E. Control Articles:
  - 1. Positive Control: none
  - 2. Negative Control: none
  - 3. Vehicle Control: none
- F. Test System: Rabbits, four females, SPF NZW, 1.5 -

2.5 kg

Source: Buckshire Corp.

P.O. Box 155

Perkasie, PA 18944

G. / Sponsor: Program Resources, Inc.

Biomedical Services Division

7655 Old Springhouse Road

McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department

Microbiological Associates, Inc.

Life Sciences Center 9900 Blackwell Road

Rockville, Maryland 20850

Animal Facility: Microbiological Associates, Inc.

5221 River Road

Bethesda, Maryland 20816

#### I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate

Study Director: Janet Luczak, M.T. (ASCP),

M.G.A.

#### J. Schedule:

1. Study Initiation Date: 07/16/90

2. Lab Initiation Date: 08/03/90

3. Lab Completion Date: 08/31/90

4. Study Completion Date: See Study Director's Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

#### L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

### III. PROCEDURES

A. Objective:

The study objective is to detect inapparent adventitious agent(s) which might be present in the test article, pre-clarified bulk live virus and/or fluids, other than the specific virus in the product.



#### B. Methods:

1. Test System Identification and Randomization

Each rabbit was housed individually. Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Each rabbit was identified by a unique number tattooed on it's ear. The rabbit's number was recorded on the cage card.

The rabbits were randomized according to SOP #OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. The rabbits were observed for 28 days for clinical signs of illness or distress.

- 3. Animal Husbandry
  - a. Rabbits were fed certified rabbit chow <u>ad</u> <u>libitum</u> and water was supplied via water bottles, <u>ad</u> <u>libitum</u>.
  - b. Rabbit's cages were changed weekly.
  - c. Animal facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

#### IV. RESULTS

All rabbits inoculated with the test article and the uninoculated control rabbit remained normal and healthy for the 28 day observation period.

See Tables 2 and 3 for a summary of the data.

#### V. CONCLUSIONS

No evidence of contamination with adventitious agent(s) due to the test article, Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7, was observed.

#### VI. **APPROVALS**

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice regulations as found in Title 21 CFR Part 58.

Mary Whiteman Mary D. Whiteman Study Director

# TABLE 1

Group	No. of Animals	Routes of Inoculation	Volume of Inoculum	Inoculum	Treatment after Inoculation
1	1	I.D. S.Q.	1.0 ml 9.0 ml	Test Article	Observe for Illness
2	1	SAA	SAA	SAA	SAA
3	1	SAA	SAA	SAA	SAA
4	1	None	None	None	SAA

SAA = Same As Above I.D. = Intradermal Inoculation

S.Q. = Subcutaneous Inoculation

TABLE 2

Survival Summary
for Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7

	RABBITS	
Test Article	3/3 <sup>a</sup>	
Uninoculated Control	1/1 <sup>b</sup>	

a Number of surviving, healthy animals after 28 days/Number of animals inoculated.

b Number of surviving, healthy animals after 28 days/Number of animals on lab initiation date.

Summary of Daily Observations
for Dengue-4 (#341756) Prod Seed, PDK-6, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post- inoc.)	Day of Death/ Sacrifice (Post- inoc.)
Rabbit	Test	11	Normal		
	Article	12	Normal		
		13	Normal		
	Uninoc- ulated				
	Control	4	Normal		

#### QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT RABBITS

Study Number: ZA359.005101

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/19/90 - 07/19/90, TO STUDY DIR 07/19/90, TO MGMT 07/19/90 PHASES: PROTOCOL REVIEW

INSPECT ON 08/16/90 - 08/16/90, TO STUDY DIR 08/16/90, TO MGMT 08/21/90 PHASES: ANIMAL OBSERVATIONS

INSPECT ON 09/21/90 - 09/24/90, TO STUDY DIR 09/24/90, TO MGMT 09/28/90 PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Ed Warburton Date
Quality Assurance Unit

## ANIMAL SAFETY TEST IN ADULT RABBITS

Study No.: ZA360.005101

Test Article: Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7

Final Report For

Program Resources, Inc. Biomedical Services Division 7655 Old Springhouse Road McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9000 Blackwell Road
Rockville, Maryland 20850

## TABLE OF CONTENTS

		Page
	Summary	34
I.	Introduction	35
II.	Study Information	35
III.	Procedures	36
IV.	Results	37
v.	Conclusions	37
VI.	Approvals	38
VII.	Ouality Assurance Statement	42



#### SUMMARY

The purpose of this assay is to detect the presence of adventitious agent(s) in the test article pre-clarified bulk live virus vaccine and/or fluids, other than the specific virus in the product. The test article was inoculated into adult rabbits.

No evidence of contamination with adventitious agent(s) was observed due to the test article Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7.

## I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent adventitious agent(s). The experimental design utilized inoculation of adult rabbits. The test is performed as described in CFR Title 21, Section 630.16.

Adult rabbits are utilized in this assay to detect possible contamination of the test article with B-virus or other adventitious agent(s) including other Simian agents, adenovirus(es), etc. which might be present in the test article. All animals are observed for signs of illness and any that become sick or show any abnormalities are examined in an attempt to establish cause of illness or death.

#### II. STUDY INFORMATION

- A. Title: Animal Safety Test in Adult Rabbits
- B. Study Number: ZA360.005101
- C. Test Article: Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7 was received at Microbiological Associates, Inc. 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.
- D. Medium Test Article: none
- E. Control Articles:
  - 1. Positive Control: none
  - 2. Negative Control: none
  - 3. Vehicle Control: none
- F. Test System: Rabbits, four females, SPF NZW, 1.5 -

2.5 kg.

Scurce: Buckshire Corp.

P.O. Box 155

Perkasie, PA 18944

G. / Sponsor: Program Resources, Inc.

Biomedical Services Division

7655 Old Springhouse Road

McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department

Microbiological Associates, Inc.

Life Sciences Center 9900 Blackwell Road

Rockville, Maryland 20850

Animal Facility: Microbiological Associates, Inc.

5221 River Road

Bethesda, Maryland 20816

#### I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate

Study Director: Janet Luczak, M.T. (ASCP),

M.G.A.

#### J. Schedule:

1. Study Initiation Date: 07/16/90

2. Lab Initiation Date: 08/03/90

3. Lab Completion Date: 08/31/90

4. Study Completion Date: See Study Director's Signature Date, in the "Approvals" Section.

## K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

#### L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

#### III. PROCEDURES

#### A. .Objective:

The study objective is to detect inapparent adventitious agent(s) which might be present in the test article, pre-clarified bulk live virus and/or fluids, other than the specific virus in the product.



#### B. Methods:

1. Test System Identification and Randomization

Each rabbit was housed individually. Each animal cage was assigned a number and labelied as "test article" or "uninoculated control". Each rabbit was identified by a unique number tattooed on it's ear. The rabbit's number was recorded on the cage card.

The rabbits were randomized according to SOP #OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. The rabbits were observed for 28 days for clinical signs of illness or distress.

- 3. Animal Husbandry
  - a. Rabbits were fed certified rabbit chow <u>ad</u>
    <u>libitum</u> and water was supplied via water
    bottles, <u>ad</u> <u>libitum</u>.
  - b. Rabbit's cages were changed weekly.
  - c. Animal facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

#### IV. RESULTS

All rabbits inoculated with the test article and the uninoculated control rabbit remained normal and healthy for the 28 day observation period.

See Tables 2 and 3 for a summary of the data.

#### V. CONCLUSIONS

No evidence of contamination with adventitious agent(s) due to the test article, Dengue-4 (#341750) Prod Seed, PDK10, FRhL-2/d7, was observed.

## VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice regulations as found in Title 21 CFR Part 58.

May D. Whiteman
Study Hirestor

Date

ΤA	~~	~	-
-11-Δ	ж,	. H"	

Group	No. of Animals	Routes of Inoculation	Volume of Inoculum	Inoculum	Treatment after <u>Inoculation</u>
1	1	I.D. S.Q.	1.0 ml 9.0 ml	Test Article	Observe for Tllness
2	1	SAA	SAA	SAA	SAA
3	1	SAA	SAA	SAA	SAA
4	1	None	None	None	SAA

SAA = Same As Above
I.D. = Intradermal Inoculation

S.Q. = Subcutaneous Inoculation

TABLE 2

Survival Summary
for Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7

	RABBITS	
Test Article	3/3 <sup>a</sup>	
Uninoculated Control	1/1 <sup>b</sup>	,

a Number of surviving, healthy animals after 28 days/Number of animals inoculated.

b Number of surviving, healthy animals after 28 days/Number of animals on lab initiation date.

TABLE 3

Summary of Daily Observations
for Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post- inoc.)	Day of Death/ Sacrifice (Post- inoc.)
Rabbit	Test Article	14 15 16	Normal Normal		
	Uninoc- ulated Control	4	Normal		

#### QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT RABBITS

Study Number: ZA360.005101

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

07/19/90 INSPECT ON 07/19/90 - 07/19/90, TO STUDY DIR 07/19/90, TO MGMT PHASES: PROTOCOL REVIEW

INSPECT ON 08/16/90 - 08/16/90, TO STUDY DIP 08/16/90, TO MGMT 08/21/90

PHASES: ANIMAL OBSERVATIONS

INSPECT ON 09/21/90 - 09/24/90, TO STUDY DIR 09/24/90, TO MGMT 09/28/90

PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

- 42 -

Er Waluton Ed Warburton

7-28-90

Date

Quality Assurance Unit

## ANIMAL SAFETY TEST IN ADULT RABBITS

Study No.: ZA361.005101

Test Article: Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7

Final Report For

Program Resources, Inc. Biomedical Services Division 7655 Old Springhouse Road McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9000 Blackwell Road
Rockville, Maryland 20850

## TABLE OF CONTENTS

		Page
	Summary	45
I.	Introduction	46
II.	Study Information	46
III.	Procedures	47
	Results	
v.	Conclusions	48
VI.	Approvals	49
VII.	Ouality Assurance Statement	53



## SUMMARY

The purpose of this assay is to attempt to detect the presence of adventitious agent(s) in the test article preclarified bulk live virus vaccine and/or fluids, other than the specific virus in the product. The test article was inoculated into adult rabbits.

No evidence of contamination with adventitious agent(s) was observed due to the test article Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7.

#### I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent adventitious agent's). The experimental design utilized inoculation of adult rabbits. The test is performed as described in CFR Title 21, Section 630.16.

Adult rabbits are utilized in this assay to detect possible contamination of the test article with B-virus or other adventitious agent(s) including other Simian agents, adenovirus(es), etc. which might be present in the test article. All animals are observed for signs of illness and any that become sick or show any abnormalities are examined in an attempt to establish cause of illness or death.

## II. STUDY INFORMATION

- A. Title: Animal Safety Test in Adult Rabbits
- B. Study Number: ZA361.005101
- C. Test Article: Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7 was received at Microbiological Associates, Inc. 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.
- D. Medium Test Article: none
- E. Control Articles:
  - 1. Positive Control: none
  - 2. Negative Control: none
  - 3. Vehicle Control: none
- F. Test System: Rabbits, four females, SPF NZW, 1.5 2.5 kg.

Source: Buckshire Corp. P.O. Box 155

Perkasie, PA 18944

G. / Sponsor: Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road

McLean, VA 22102

Authorized Representative: Dr. Louis Potash



H. Testing Facility: Biotechnology Services Department

Microbiological Associates, Inc.

Life Sciences Center 9900 Blackwell Road

Rockville, Maryland 20850

Animal Facility: Microbiological Associates, Inc.

5221 River Road

Bethesda, Maryland 20816

#### I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate

Study Director: Janet Luczak, M.T. (ASCP),

M.G.A.

#### J. Schedule:

1. Study Initiation Date: 07/16/90

2. Lab Initiation Date: 08/03/90

3. Lab Completion Date: 08/31/90

4. Study Completion Date: See Study Director's Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

#### L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

#### III. PROCEDURES

## A. -Objective:

The study objective is to detect inapparent adventitious agent(s) which might be present in the test article, pre-clarified bulk live virus and/or fluids, other than the specific virus in the product.

#### B. Methods:

1. Test System Identification and Randomization

Each rabbit was housed individually. Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Each rabbit was identified by a unique number tattooed on it's ear. The rabbit's number was recorded on the cage card.

The rabbits were randomized according to SOP #OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. The rabbits were observed for 28 days for clinical signs of illness or distress.

- 3. Animal Husbandry
  - a. Rabbits were fed certified rabbit chow <u>ad</u>
    <u>libitum</u> and water was supplied via water
    bottles, <u>ad</u> <u>libitum</u>.
  - b. Rabbit's cages were changed weekly.
  - c. Animal facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

## IV. RESULTS

All rabbits inoculated with the test article and the uninoculated control rabbit remained normal and healthy for the 28 day observation period.

See Tables 2 and 3 for a summary of the data.

#### V. CONCLUSIONS

No evidence of contamination with adventitious agent(s) due to the test article, Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7, was observed.

## VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice Regulations as found in Title 21 CFR Part 58.

May D. Whiteman
Study Director

Date

9/27/90

## TABLE 1

Group	No. of Animals	Routes of Inoculation	Volume of Inoculum	Inoculum	Treatment after Inoculation
1.	1	I.D. S.Q.	1.0 ml 9.0 ml	Test Article	Observe for Illness
2	1	SAA	SAA	SAA	SAA
3	1	SAA	SAA	SAA	SAA
4	1	None	None	None	SAA

SAA = Same As Above
I.D. = Intradermal Inoculation S.Q. = Subcutaneous Inoculation

TABLE 2 Survival Summary for Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7

	RABBITS		
Test Article	3/3 <sup>a</sup>		
Uninoculated Control	1/1 <sup>b</sup>		

a Number of surviving, healthy animals after 28 days/Number of

animals inoculated.

b Number of surviving, healthy animals after 28 days/Number of animals on lab initiation date.

TABLE 3

Summary of Daily Observations
for Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post- inoc.)	Day of Death/ Sacrifice (Post- inoc.)
Rabbit	Test Article	17 18 19	Normal Normal Normal		
	Uninoc- ulated Control	4	Normal		

#### QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT RABBITS

Study Number: ZA361.005101

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/19/90 - 07/19/90, TO STUDY DIR 07/19/90, TO MGMT 07/19/90 PHASES: PROTOCOL REVIEW

INSPECT ON 08/16/90 - 08/16/90, TO STUDY DIR 08/16/90, TO MGMT 08/21/90 PHASES: ANIMAL OBSERVATIONS

INSPECT ON 09/21/90 - 09/24/90, TO STUDY DIR 09/24/90, TO MGMT 09/28/90 PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Ed Warburton

9-28-60

Quality Assurance Unit

Date

## APPENDIX

С

# ANIMAL SAFETY TEST IN ADULT MICE AND SUCKLING MICE

Study NO.: ZA359.005100						
Dengue-4 Prod Seed: PDK- 6,	FRhL-2/d7		pages	55		67
Study NO.: ZA360.005100						
Dengue-4 Prod Seed: PDK-10,	FRhL-2/d7	•••••	pages	68	-	81
Study NO.: ZA361.005100						
Dengue_4 Prod Seed: PDK_15	FRbI -2/d7		nages	82	_	gg

## ANIMAL SAFETY TEST IN ADULT MICE AND SUCKLING MICE

Study No.: ZA359.005100

Test Article: Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7

Final Report For

Program Resources, Inc. Biomedical Services Division 7655 Old Springhouse Road McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

## ZA359.005100

## TABLE OF CONTENTS

	· ·	Page
	Summary	57
I.	Introduction	58
II.	Study Information	58
ŢΙΙ.	Procedures	60
IV.	Results	61
ν.	Conclusions	62
VI.	Approvals	62
VII.	Quality Assurance Statement	67

#### SUMMARY

The purpose of this assay is to detect the presence of adventitious agent(s) in the test article, pre-clarified bulk live virus vaccines and/or fluids, other than the specific virus in the product. The test article was inoculated into adult mice and suckling mice. The suckling mouse portion of the assay included a subpassage of homogenized tissue after 14 days into a new group of suckling mice, followed by an additional 14 day observation period.

No evidence of contamination with adventitious agents due to the test article Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7 was observed.

#### I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent virus(es). The experimental design utilizes inoculations of adult and suckling mice. The test is performed as described in CFR Title 21, Section 630.35 (a)(1)(2).

Adult mice are included in the assay to detect possible contamination of the test article with neurotropic or other viruses such as lymphocytic choriomeningitis virus. Suckling mice are used to detect Coxsackie or other viruses. All animals are observed for signs of illness and any that become sick or show any abnormalities are examined in an attempt to establish cause of illness or death.

## II. STUDY INFORMATION

- A. Title: Animal Safety Test in Adult Mice and Suckling Mice
- B. Study Number: ZA359.005100
- C. Test Article: Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7 was received at Microbiological Associates, Inc. on 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.
- D. Medium Test Article: none
- E. Control Articles:
  - 1. Positive Control: none
  - 2. Negative Control: none
  - 3. Vehicle Control: none
- F. Test System: Mice

Suckling litters (Primary Inoculation): Tac(SW)fBR, three adult females each with ten <24 hour old suckling pups,

, Source: Taconic Farms, Germantown, New York

Suckling litters (Blind Passage): Tac: (SW) fBR, four adult females each with ten <24 hour old suckling pups,

Source: Taconic Farms, Germantown, New York



#### ZA359.005100

Adult - HSD:ICR, Fifteen males and fifteen females, Body Weight range: 15-20 grams. Source: Harlan Sprague Dawley, Frederick, Maryland

G. Sponsor: Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

Animal Facility: Microbiological Associates, Inc. 5221 River Road

Bethesda, Maryland 20816

## I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate Study Director: Janet Luczak, M.T. (ASCP), M.G.A.

## J. Schedule:

1. Study Initiation Date: 07/16/90

2. Lab Initiation Date: 07/24/90

3. Lab Completion Date: 08/23/90

4. Study Completion Date: See Study Director's Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of , samples of the test article is the responsibility of the sponsor.

#### ZA359.005100

#### L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

#### III. PROCEDURES

With approval of the sponsor, a previously thawed test article sample which was frozen back and stored at -70°C ± 10°C was utilized in the inoculation of the suckling mouse portion of the assay. In addition, at the request of the sponsor, in the suckling mouse portion of the assay, 1.3 ml of the test article was combined with 1.3 ml of sponsor supplied antisera Den-4 #814669 Carec, SM5 of 11-21-82 and heated at 37°C for 90 minutes, prior to inoculation of the suckling mice. The remaining untreated sample was again frozen back and was utilized along with a previously unthawed aliquot of the test article to inoculate the adult mouse portion of the assay.

## A. Objective:

The study objective is to detect inapparent virus(es) that might be present in the test article, preclarified bulk live virus and/or fluids, other than the specific virus in the product.

#### B. Methods:

1. Test System Identification and Randomization

Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Adult mice were ear-tagged but housed in groups according to inoculum type and sex. Suckling mice were not individually identified.

Suckling and adult mice were randomized according to SOP #OPBT0213.

#### 2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. The adult mice were observed every working day for 28 days for clinical signs. In the suckling mouse portion of the assay, the animals were inoculated according to Table 1 and were then observed every working day for 14 days for clinical signs. Fourteen days post-inoculation, all surviving



suckling mice from each group were euthanized using cervical dislocation. Following euthanasia their skin and gastrointestine were removed, the carcasses cut into pieces and placed in a sterile pre-weighed bowl. After determining the weight of the entire group of mice from a cage, enough Hank's Balanced Salt Solution (HBSS) with gentamicin was added to make a 20% w/v suspension. The entire content of the bowl was then homogenized in a sterile blender, clarified by centrifugation, diluted 1:2 in HBSS and subsequently inoculated into a new group of suckling mice by the same routes and in the same volumes as the original group. These newly inoculated mice were observed for a period of fourteen days. Ten suckling mice were held as uninoculated controls and observed for fourteen davs.

## 3. Animal Husbandry

a. All animals were fed the following diet ad libitum:

Mice - autoclavable chow.

- b. Water was supplied ad libitum via fresh apples.
- c. Bedding Bed-o-Cobs, Anderson Cob Mills. Cages were changed as necessary, usually twice per week.
- d. Animal facilities are accredited by the American Association for Accreditation of Laboratory Animal Care.

#### IV. RESULTS

All adult mice inoculated with the test article and all uninoculated control adult mice remained normal and healthy for the twenty-eight day observation period.

All uninoculated control suckling mice and test article inoculated suckling mice appeared normal and healthy for the 14 day observation period. The surviving mice of each group were homogenized and the homogenate of each group was passaged into a new group of suckling mice. The remainder of the homogenates was frozen at  $-70^{\circ}\text{C} \pm 10^{\circ}\text{C}$ .



#### ZA359.005100

In the blind passage all of the uninoculated control suckling mice, all of the suckling mice inoculated with the homogenate of the uninoculated control suckling mice, and nineteen of the twenty suckling mice inoculated with the homogenate of the test article inoculated suckling mice appeared normal and healthy for the 14 day observation period. One of the test article homogenate inoculated suckling mice was missing and presumed cannibalized day four post-inoculation.

See Tables 2 and 3 for a summary of the data.

#### V. CONCLUSIONS

No evidence of contamination with adventitious agents was observed due to the test article, Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7, was observed.

#### VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice Regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman
Study Director

Date

TABLE 1

Group #	Number of Animals	Sex	Species	Route(s) of Inoculation	Volume of Inoculum	Inoculum	Treatment after Inoculation
SM-1	1	female	mouse (lactating)	None	Nonr	None	None
	+						
	10	various	Mouse (suckling)	i.p.	0.1 ml 0.01 ml	test article test article	Observed for illness after 14 days passage a single pool of emulsified tissue (minus skin and gastrointestine) of all surviving mice onto at least 10 additional suckling mice. Use same routes and volumes as original
SM 2	SAA*	SAA	SAA	SAA	SAA	SAA	SAA
SN 3	SAA	SAA	SAA	None	None	uninoc control	SAA
AM 1	5	male	mouse	i.p. i.c.	0.5 ml 0.03 ml	test article	Ob⊛⊃rye for illness
AH 2	5	male	AĀc	SAA	SAA	SAA	SAA
AM 3	5	female	SAA	SAA	SAA	SAA	SAA
AH 4	5	female	SAA	SAA	SAA	SAA	SAA
AM 5	5	male	SAA	None	None	uninoc control	SAA
AM 6	5	female	SAA	None	None	SAA	SAA

SAA = Same as above

i.c. = Intracranial

i.p. = Intraperitoneal

TABLE 2

Survival Summary

for Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7

		Suckling Miceb		
	Adult Mice <sup>a</sup>	Primary Inoculation	Blind Passage	
Test Article	20/20	20/20	19/20	
Uninoculated Control	10/10	10/10	10/10	
Uninoculated Control <sup>C</sup>			10/10	

a Number of surviving, healthy animals after 28 days/Number of animals inoculated.

b In the suckling mice portion of the assay, animals are inoculated and observed for 14 days. On day 14 post-inoculation a homogenate is repared from the surviving sucklings from each group. This homogenate was used to inoculate another group of suckling mice which were observed for an additional 14 days. The number of surviving sucklings/number of animals inoculated is presented.

In the blind passage of the suckling mouse portion of the assay an uninoculated control group was held with the blind passage animals inoculated with the homogenate of the test article and the homogenate of the uninoculated control group from the primary inoculation.

TABLE 3

Summary of Daily Observations
for Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post- inoc.)	Day of Death/ Sacrifice (Post- inoc.
Adult Mice	Test Article	10601 10602 10603 10604 10605 10606 10607 10608 10609 10610 10611 10612 10613 10614 10615 10616 10617 10618 10619 10620	Normal		
	Uninoc- ulated Control	10621 10622 10623 10624 10625 10626 10627 10628 10629 10630	Normal		



TABLE 3 (Cont.)

Summary of Daily Observations
for Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7

Animal Species	Inoculum	Numk Cage	per/ ga	Clinical Signs	Day of Onset (Post- Inoc.)	Day of Death/ Sacrifice (Post- Inoc.)
Sucklingb Mice (Primary Inoculation)	Test Article		(10) (10)	Normal Normal		
	Uninocu- lated Control	SM3	(10)	Normal		
(Blind Passage)	Test Article Homo- genate		(10) (9)° (10)	Normal Normal	4	4
	Uninocu- lated Control Homo- genate		(10)	Normal		
	Uninocu- lated Control		(10)	Normal		

a Ten suckling mice inoculated per cage.



b Surviving suckling mice from primary inoculation were sacrificed on day 14 for preparation of blind passage tissue homogenate.

<sup>&</sup>lt;sup>C</sup> One suckling mouse was missing and presumed cannibalized.

#### QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT MICE AND SUCKLING MICE

Study Number: ZA359.005100

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPS (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/19/90 - 07/19/90, TO STUDY DIR 07/19/90, TO MGMT 07/19/90 PHASES: PROTOCOL REVIEW

INSPECT ON 08/23/90 - 08/23/90, TO STUDY DIR 08/23/90, TO MGMT 08/23/90 PHASES: ANIMAL OBSERVATIONS

INSPECT ON 10/17/90 - 10/17/90, TO STUDY DIR 10/17/90, TO MGMT 10/25/90 PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Dana H. Hamblen

Quality Assurance Unit

Date

# ANIMAL SAFETY TEST IN ADULT MICE AND SUCKLING MICE

Study No.: ZA360.005100

Test Article: Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7

Final Report For

Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

### TABLE OF CONTENTS

		Page
	Summary	70
I.	Introduction	71
II.	Study Information	71
III.	Procedures	73
IV.	Results	74
v.	Conclusions	75
VI.	Approvals	76
VII.	Quality Assurance Statement	81

### SUMMARY

The purpose of this assay is to attempt to detect the presence of adventitious agent(s) in the test article, preclarified bulk live virus vaccines and/or fluids, other than the specific virus in the product. The test article was inoculated into adult mice and suckling mice. The suckling mouse portion of the assay included a subpassage of homogenized tissue after 14 days into a new group of suckling mice, followed by an additional 14 day observation period.

No evidence of contamination with adventitious agents due to the test article Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7 was observed in the adult mouse portion of the assay. The results of the suckling mouse portion of the assay were inconclusive due to the lethal effect of the specific virus in the product, on the suckling mice.

### I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent virus(es). The experimental design utilizes inoculations of adult and suckling mice. The test is performed as described in CFR Title 21, Section 630.35 (a)(1)(2).

Adult mice are included in the assay to detect possible contamination of the test article with neurotropic or other viruses such as lymphocytic choriomeningitis virus. Suckling mice are used to detect Coxsackie or other viruses. All animals are observed for signs of illness and any that become sick or show any abnormalities are examined in an attempt to establish cause of illness or death.

### II. STUDY INFORMATION

- A. Title: Animal Safety Test in Adult Mice and Suckling Mice
- B. Study Number: ZA360.005100
- C. Test Article: Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7 was received at Microbiological Associates, Inc. on 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.
- D. Medium Test Article: none
- E. Control Articles:
  - 1. Positive Control: none
  - 2. Negative Control: none
  - 3. Vehicle Control: none
- F. Test System: Mice

Suckling litters (Primary Inoculation): Tac:(SW)fBR, three adult females each with ten <24 hour old suckling pups,

, Source: Taconic Farms, Germantown, New York

Suckling litters (Blind Passage): Tac:(SW)fBR, four adult females each with ten <24 hour old suckling pups,

Source: Taconic Farms, Germantown, New York



### ZA360.005100

Adult - HSD:ICR, Fifteen males and fifteen females, Body Weight range: 15-20 grams. Source: Harlan Sprague Dawley, Frederick, Maryland

G. Sponsor: Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean. VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

Animal Facility: Microbiological Associates, Inc. 5221 River Road
Bethesda, Maryland 20816

### I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate
Study Director: Janet Luczak, M.T. (ASCP), M.G.A.

### J. Schedule:

1. Study Initiation Date: 07/16/90

2. Lab Initiation Date: 07/24/90

3. Lab Completion Date: 08/23/90

4. Study Completion Date: See Study Director's Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of , samples of the test article is the responsibility of the sponsor.

### ZA360.005100

### L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

### III. PROCEDURES

With approval of the sponsor, a previously thawed test article sample which was frozen back and stored at  $-70^{\circ}\text{C} \pm 10^{\circ}\text{C}$  was utilized in the inoculation of the suckling mouse portion of the assay. In addition, at the request of the sponsor, in the suckling mouse portion of the assay, 1.3 ml of the test article was combined with 1.3 ml of sponsor supplied antisera Den-4 #814669 Carec, SM5 of 11-21-82 and heated at  $37^{\circ}\text{C}$  for 90 minutes, prior to inoculation of the suckling mice. The remaining untreated sample was again frozen back and was utilized along with a previously unthawed aliquot of the test article to inoculate the adult mouse portion of the assay.

### A. Objective:

The study objective is to detect inapparent virus(es) that might be present in the test article, preclarified bulk live virus and/or fluids, other than the specific virus in the product.

### B. Methods:

1. Test System Identification and Randomization

Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Adult mice were ear-tagged but housed in groups according to inoculum type and sex. Suckling mice were not individually identified.

Suckling and adult mice were randomized according to SOP #OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. The adult mice were observed every working day for 28 days for clinical signs. In the suckling mouse portion of the assay, the animals were inoculated according to Table 1 and were then observed every working day for 14 days for clinical signs.



Fourteen days post-inoculation, all surviving suckling mice from each group were euthanized using cervical dislocation. Following euthanasia their skin and gastrointestine were removed, the carcasses cut into pieces and placed in a sterile pre-weighed bowl. After determining the weight of the entire group of mice from a cage, enough Hank's Balanced Salt Solution (HBSS) with gentamicin was added to make a 20% w/v suspension. The entire content of the bowl was then homogenized in a sterile blender, clarified by centrifugation, diluted 1:2 in HBSS and subsequently inoculated into a new group of suckling mice by the same routes and in the same volumes as the original group. These newly inoculated mice were observed for a period of fourteen days. Ten suckling mice were held as uninoculated controls and observed for fourteen days.

### 3. Animal Husbandry

a. All animals were fed the following diet  $\underline{ad}$  libitum:

Mice - autoclavable chow.

- b. Water was supplied ad <u>libitum</u> via fresh apples.
- c. Bedding Bed-o-Cobs, Anderson Cob Mills. Cages were changed as necessary, usually twice per week.
- d. Animal facilities are accredited by the American Association for Accreditation of Laboratory Animal Care.

### IV. RESULTS

All adult mice inoculated with the test article and all uninoculated control adult mice remained normal and healthy for the twenty-eight day observation period.

All of the uninoculated control suckling mice and nineteen of the twenty test article inoculated suckling mice appeared normal and healthy for the 14 day observation period. One of the test article inoculated suckling mice appeared lethargic and had a swollen head day 14 post-inoculation, prior to sacrifice of all surviving mice of



each group. The surviving mice were homogenized and the homogenate of each group was passaged into a new group of suckling mice. The remainder of the homogenates was frozen at  $-70^{\circ}\text{C} \pm 10^{\circ}\text{C}$ .

In the blind passage, all of the uninoculated control suckling mice, all of the suckling mice inoculated with the homogenate of the uninoculated control suckling mice and seven of the twenty suckling mice inoculated with the homogenate of the test article inoculated suckling mice appeared normal and healthy for the 14 day observation Two of the suckling mice inoculated with the homogenate of the test article inoculated suckling mice were found partially cannibalized; one on day 9 postinoculation and one on day 10 post-inoculation. Four additional suckling mice in the test article homogenate group were moribund day 10 post-inoculation and were sacrificed. Necropsy was performed. Four of the test article homogenate inoculated suckling mice were found dead day 12 post-inoculation. One of the test article homogenate inoculated suckling mice appeared lethargic day 13 post-inoculation and was found partially cannibalized day 14 post-inoculation. Of the remaining suckling mice, two appeared lethargic on day 14 post-inoculation. requested by the sponsor that no histopathologic examination be performed on the surviving, dead, or moribund suckling mice. The death of the suckling mice was most likely caused by the specific virus in the product, and was not an unexpected response to the intracranial inoculation of suckling mice with this product.

See Tables 2 and 3 for a summary of the data.

### V. CONCLUSIONS

No evidence of contamination with adventitious agent(s) due to the test article, Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7, was observed in the adult mouse portion of the assay. The results of the suckling mouse portion of the assay were inconclusive due to the lethal effect of the specific virus in the product, on the suckling mice.



### ZA360.005100

### VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice Regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman 10/23/90
Date

Study Mirector

MICROBIOLOGICAL ASSOCIATES, INC.

TABLE 1

Group #	Number of Animals	Sex	Species	Route(s) of Inoculation	Volume of   Inoculum	Inocultm	Treatment after Inoculation
SM 1	1	female	mouse (lactating)	None	None	None	None
	+ 10	various	Mouse (suckling)	i.p.	0.1 ml 0.01 ml	test article test article	Observed for illness after 14 days passage a single pool of emulsified tissue (minus skin and gastrointestine) of all surviving mice onto at least 10 additional suckling mice. Use same routes and volumes as original.
SM 2	SAA*	SAA	SAA	SAA	SAA	SAA	SAA-
SM 3	SAA	SAA	SAA	None	None	uninoc control	SAA
AM 1	5	male	mouse	i.p. i.c.	0.5 ml 0.03 ml	test article	Observe for illness
S MA	5	male	SAA	SAA	SAA	SAA	SAA
AM 3	5	female	SAA	SAA	SAA	SAA	SAA
AM 4	5	female	SAA	SAA	SAA	SAA	SAA
AM 5	5	male	SAA	None	None	uninoc control	SAA
AM 6	5	female	SAA	None	None	SAA	SAA

SAA = Same as above

i.c. = Intracranial

i.p. = Intraperitoneal

.



TABLE 2

Survival Summary

for Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7

		Suckling	Miceb
	Adult Mice <sup>a</sup>	Primary Inoculation	Blind Passage
Test Article	20/20	20/20	9/20
Uninoculated Control	10/10	10/10	10/10
Uninoculated Control <sup>C</sup>			10/10

<sup>&</sup>lt;sup>a</sup> Number of surviving, animals after 28 days/Number of animals inoculated.

b In the suckling mice portion of the assay, animals are inoculated and observed for 14 days. On day 14 post-inoculation a homogenate is prepared from the surviving sucklings from each group. This homogenate was used to inoculate another group of suckling mice which were observed for an additional 14 days. The number of surviving sucklings/number of animals inoculated is presented.

<sup>&</sup>lt;sup>C</sup> In the blind passage of the suckling mouse portion of the assay an uninoculated control group was held with the blind passage animals inoculated with the homogenate of the test article and the homogenate of the uninoculated control group from the primary inoculation.

TABLE 3

Summary of Daily Observations
for Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post- inoc.)	Day of Death/ Sacrifice (Post- inoc.
Adult Mice	Test Article	10631 10632 10633 10634 10635 10636 10637 10638 10640 10641 10642 10643 10644 10645 10646 10647 10648 10649 10650	Normal		
	Uninoc- ulated Control	10621 10622 10623 10624 10625 10626 10627 10628 10629 10630	Normal		·

TABLE 3 (Cont.) Summary of Daily Observations for Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7

Animal Species	Inoculum	Numb Cage		Clinical Signs	Day of Onset (Post- Inoc.)	Day of Death/ Sacrifice (Post- Inoc.)
Suckling <sup>b</sup> Mice (Primary Inoculation)	Test Article	SM1 SM2	(10) (10) <sup>g</sup> (10)	Normal Normal	14	
	Uninocu- lated Control	SM3	(10)	Normal		
(Blind Passage)	Test Article Homo- genate	SM1	(10) (9)c (8)c (4)d (0)e (10) (9)f (9)h	Normal Normal	9 10 10 12 13 14	9 10 10 12 14
	Uninocu- lated Control Homo- genate	SM3	(10)	Normal		
	Uninocu- lated Control	SM4	(10)	Normal		

a Ten suckling mice inoculated per cage.

e Four suckling mice were found dead.

f One suckling mouse appeared runted and hunched day 13 postinoculation and was found partially cannibalized day 14.

9 One suckling mouse was lethargic and appeared to have a swollen head.

h Two suckling mice were lethargic and had hunched postures.

MICROBIOLOGICAL ASSOCIATES, INC.

b Surviving suckling mice from primary inoculation were sacrificed on day 14 for preparation of blind passage tissue homogenate.

C One suckling mouse was found partially cannibalized.

d Four suckling mice appeared moribund and were sacrificed. Necropsy was performed.

### QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT MICE AND SUCKLING MICE

Study Number: ZA360.005100

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedues, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/19/90 - 07/19/90, TO STUDY DIR 07/19/90, TO MGMT 07/19/90 PHASES: PROTOCOL REVIEW

INSPECT ON 08/23/90 - 08/23/90, TO STUDY DIR 08/23/90, TO MGMT 08/23/90 PHASES: ANIMAL OBSERVATIONS

INSPECT ON 10/17/90 - 10/17/90, TO STUDY DIR 10/17/90, TO MGMT 10/25/90 PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Dana H./Hamblen

Mun

Quality Assurance Unit

Date

## ANIMAL SAFETY TEST IN ADULT MICE AND SUCKLING MICE

Study No.: ZA361.005100

Test Article: Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7

Final Report For

Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

### ZA361.005100

7

### TABLE OF CONTENTS

		Page
	Summary	84
I.	Introduction	85
II.	Study Information	85
III.	Procedures	87
IV.	Results	89
v.	Conclusions	90
VI.	Approvals	90
VII.	Quality Assurance Statement	96
IX.	Appendix	97

### SUMMARY

The purpose of this assay is to attempt to detect the presence of adventitious agent(s) in the test article, preclarified bulk live virus vaccines and/or fluids, other than the specific virus in the product. The test article was inoculated into adult mice and suckling mice. The suckling mouse portion of the assay included a subpassage of homogenized tissue after 14 days into a new group of suckling mice, followed by an additional 14 day observation period.

No evidence of contamination with adventitious agents due to the test article Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7 was observed in the adult mouse portion of the assay. The results of the suckling mouse portion of the assay were inconclusive, due to the lethal effect of the specific virus in the product on the suckling mice.

### ZA361.005100

### I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent adventitious agent(s). The experimental design utilizes inoculations of adult and suckling mice. The test is performed as described in CFR Title 21, Section 630.35 (a)(1)(2).

Adult mice are included in the assay to detect possible contamination of the test article with neurotropic or other viruses such as lymphocytic choriomeningitis virus. Suckling mice are used to detect Coxsackie or other viruses. All animals are observed for signs of illness and any that become sick or show any abnormalities are examined in an attempt to establish cause of illness or death.

### II. STUDY INFORMATION

- A. Title: Animal Safety Test in Adult Mice and Suckling Mice
- B. Study Number: ZA361.005100
- C. Test Article: Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7 was received at Microbiological Associates, Inc. on 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.
- D. Medium Test Article: none
- E. Control Articles:
  - 1. Positive Control: none
  - 2. Negative Control: none
  - 3. Vehicle Control: none
- F. Test System: Mice

Suckling litters (Primary Inoculation): Tac:(SW)fBR, three adult females each with at least eight <24 hour old suckling pups,

/ Source: Taconic Farms, Germantown, New York

Suckling litters (Blind Passage): Tac(SW)fBR, six adult females each with ten <24 hour old suckling pups,

Source: Taconic Farms, Germantown, New York

Adult - HSD: ICR, Fifteen males and fifteen females,

Body Weight range: 15-20 grams.

Source: Harlan Sprague Dawley, Frederick, Maryland

G. Sponsor: Program Resources, Inc. Biomedical Services Division 7655 Old Springhouse Road McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department Microbiological Associates, Inc. Life Sciences Center 9900 Blackwell Road

Rockville, Maryland 20850

Microbiological Associates, Inc. Animal Facility:

5221 River Road

Bethesda, Maryland 20816

#### I. Personnel:

Study Director: Mary D. Whiteman

2. Associate

Study Director: Janet Luczak, M.T. (ASCP), M.G.A.

#### Schedule: J.

Study Initiation Date: 07/16/90

2. Lab Initiation Date: 07/24/90

3. Lab Completion Date: 08/28/90

4. Study Completion Date: See Study Director's

Signature Date, in the

"Approvals" Section.

K., Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of

### ZA361.005100

samples of the test article is the responsibility of the sponsor.

### L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

### III. PROCEDURES

With approval of the sponsor, a previously thawed test article sample which was frozen back and stored at  $-70^{\circ}$ C  $\pm$ 10°C was utilized in the inoculation of the suckling mouse portion of the assay. In addition, the sponsor requested that in the suckling mouse portion of the assay 1.3 ml of the test article be combined with 1.3 ml of the sponsor supplied antisera Den-4 #814669 Carec, SM5 of 11-21-82 and heated at 37°C for 90 minutes prior to inoculation of the suckling mice. This procedure was followed except that due to an insufficient volume of antisera, 1.2 ml of antisera was combined with 1.2 ml of the test article. This procedural change was approved by the sponsor. remaining untreated sample was frozen back and was utilized along with a previously unthawed aliquot of the test article to inoculate the adult mouse portion of the assay.

### A. Objective:

The study objective is to detect inapparent virus(es) that might be present in the test article, preclarified bulk live virus and/or fluids, other than the specific virus in the product.

### B. Methods:

1. Test System Identification and Randomization

Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Adult mice were ear-tagged but housed in groups according to inoculum type and sex. Suckling mice were not individually identified.

Suckling and adult mice were randomized according to SOP #OPBT0213.



### 2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. The adult mice were observed every working day for 28 days for clinical signs. In the suckling mouse portion of the assay, the animals were inoculated according to Table 1 and were then observed every working day for 14 days for clinical signs.

Note: After preparation of a 1:1 dilution of the remaining antisera Den-4 #814669 Carec, SM5 of 11-21-82 and the test article, the resulting volume was not sufficient to inoculate 10 suckling mice per group. After consultation with the sponsor, and with the sponsors approval, eight suckling mice were inoculated per group.

Fourteen days post-inoculation, all surviving suckling mice from each group were euthanized using cervical dislocation. Following euthanasia their skin and gastrointestine were removed, the carcasses cut into pieces and placed in a sterile pre-weighed bowl. After determining the weight of the entire group of mice from a cage, enough Hank's Balanced Salt Solution (HBSS) with gentamycin was added to make a 20% w/v suspension. The entire content of the bowl was then homogenized in a sterile blender, clarified by centrifugation, diluted 1:2 in HBSS and subsequently inoculated into a new group of suckling mice by the same routes and in the same volumes as the original group. These newly inoculated mice were observed for a period of fourteen days. Ten suckling mice were held as uninoculated controls and observed for fourteen days.

### 3. Animal Husbandry

a. All animals were fed the following diet <u>ad</u> libitum:

Mice - autoclavable chow.

- b. Water was supplied <u>ad libitum</u> via fresh apples.
- c. Bedding Bed-o-Cobs, Anderson Cob Mills. Cages were changed as necessary, usually twice per week.



d. Animal facilities are accredited by the American Association for Accreditation of Laboratory Animal Care.

### IV. RESULTS

All adult mice inoculated with the test article and all uninoculated control adult mice remained normal and healthy for the twenty-eight day observation period.

All of the uninoculated control suckling mice and six of the sixteen test article inoculated suckling mice appeared normal and healthy for the 14 day observation period. One of the test article inoculated suckling mice was missing and presumed cannibalized day one post-inoculation. On day 13 post-inoculation, five of the test article inoculated suckling mice appeared moribund and were sacrificed and homogenized for passage into new groups of suckling mice. Four of the test article inoculated suckling mice were found dead; three on day 13 and one on day 14 post-inoculation. Necropsy and histopathology were performed on the dead suckling mice. The cause of death of these animal could not be determined due to the degree of autolysis in the tissues examined. (See Pathology Report in Appendix.)

In the blind passage, all of the uninoculated control suckling mice and all of the suckling mice inoculated with the homogenate of the uninoculated control suckling mice appeared normal and healthy for the 14 day observation period. One of the test article homogenate inoculated suckling mice was missing and presumed cannibalized day 9 post-inoculation. By day 10 post-inoculation, all but one of the remaining suckling mice appeared lethargic and hunched and were staggering. On day 11 post-inoculation, one test article homogenate inoculated suckling mouse was found dead. Eight test article homogenate inoculated suckling mice were found dead day 12 post-inoculation. Two of the dead suckling mice were partially cannibalized. On day 13 post-inoculation, of the ten remaining test article homogenate inoculated suckling mice, five were found dead, one was found partially cannibalized and four Two of the moribund suckling mice were moribund. inoculated with the test article homogenate, were sacrificed. Necropsy was performed. On day 14 postinoculation, the remaining two suckling mice were found dead.



### ZA361.005100

Of the twenty suckling mice inoculated with the homogenates of the test article inoculated mice which were moribund day 13 post-inoculation, eight appeared normal and healthy for the 14 day observation period. Two of the suckling mice inoculated with the homogenate of the test article moribund sucklings were missing and presumed cannibalized day 1 post-inoculation and one was missing and presumed cannibalized day 4 post-inoculation. Of the seventeen remaining suckling mice inoculated with the homogenate of the moribund test article suckling mice, on day 13 post-inoculation, two were found partially cannibalized, four were moribund and one was runted and lethargic. One of the four moribund suckling mice and the runted suckling mouse were sacrificed. Necropsy was performed. On day 14 post-inoculation, three suckling mice were found dead and two were moribund.

It was requested by the sponsor that no histopathologic examination be performed on the dead, sacrificed or moribund suckling mice. The death of the suckling mice was most likely caused by the specific virus in the product and was not an unexpected response to the intracranial inoculation of suckling mice with this product.

See Tables 2 and 3 for a summary of the data.

### V. CONCLUSIONS

No evidence of contamination with adventitious agents due to the test article, Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7, was observed in the adult mouse portion of the assay. The results of the suckling mouse portion of the assay were inconclusive due to the lethal effect of the specific virus in the product on the suckling mice.

### VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice Regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman

Study Director

70/34/30 Date



TABLE 1

Group #	Number of Animals	Sex	Species	Route(s) of Inoculation	Volume of	Inoculum	Treatment after Inoculation
SM 1	1	female	imuse (lactating)	None	None	None	None
	+ 10	various	Mouse (suckling)	í.p. i.c.	0.1 ml 0.01 ml	test article test article	Observed for illness after 14 days passage a single pool of emulsified tissue (minus skin and gastrointestine) of all surviving mice onto at least 10 additional suckling mice. Use same routes and volumes as original.
SM 2	SAA*	SAA	SAA	SAA	SAA	SAA	SAA
SM 3	SAA	SAA	SAA	None	None	uninoc control	SAA
AM 1	5	male	mouse	i.p.	0.5 ml 0.03 ml	test article	Observe for illness
AM 2	5	male	SAA	SAA	SAA	SAA	SAA
AM 3	5	female	SAA	SAA	SAA	SAA	SAA
AM 4	5	female	SAA	SAA	SAA	SAA	SAA
AM 5	5	male	SAA	None	None	uninoc control	SAA
АМ 6	5	female	SAA	None	None	SAA	SAA

SAA = Same as above

i.c. = Intracranial

i.p. = Intraperitoneal



TABLE 2

Survival Summary

for Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7

		Suckling N	Mice <sup>b</sup>
	Adult Mice <sup>a</sup>	Primary Inoculation	Blind Passage
Test Article	20/20	6/16	0/20
Uninoculated Control	10/10	10/10	10/10
Uninoculated Control <sup>C</sup>			10/10
Test Article Homogenate of Moribund			
Sucklings			10/20

a Number of surviving, animals after 28 days/Number of animals inoculated.

b In the suckling mice portion of the assay, animals are inoculated and observed for 14 days. On day 14 post-inoculation a homogenate is prepared from the surviving sucklings from each group. This homogenate was used to inoculate another group of suckling mice which were observed for an additional 14 days. The number of surviving sucklings/number of animals inoculated is presented.

<sup>&</sup>lt;sup>C</sup> In the blind passage of the suckling mouse portion of the assay an uninoculated control group was held with the blind passage animals inoculated with the homogenate of the test article and the homogenate of the uninoculated control group from the primary inoculation.

TABLE 3

Summary of Daily Observations
for Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post- inoc.)	Day of Death/ Sacrifice (Post- inoc.
Adult Mice	Test Article	10651 10652 10653 10654 10655 10656 10657 10658 10669 10661 10662 10663 10664 10665 10666 10667 10668 10669 10670	Normal		
	Uninoc- ulated Control	10621 10622 10623 10624 10625 10626 10627 10628 10629 10630	Normal		

TABLE 3 (Cont.)

Summary of Daily Observations
for Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7

Animal Species	Inoculum	Number/ Cage <sup>a</sup>	Clinical Signs	Day of Onset (Post- Inoc.)	Day of Death/ Sacrifice (Post- Inoc.)
Suckling <sup>b</sup> Mice (Primary Inoculation)	Test Article	SM1 (8) (6)f (3)g (2)d SM2 (8) (7)c (6)d (4)e	Normal Normal	13 13 14 1 1 13 13	13 13 14 1 13 13
	Uninocu- lated Control	SM3 (10)	Normal		

a Eight to ten suckling mice inoculated per cage.

One suckling mouse missing and presumed cannibalized.

e Two moribund suckling mice were homogenized for passage.

f Two suckling mice found dead and sent for necropsy and histopathology.

9 Three moribund suckling mice were homogenized for passage.

b Surviving suckling mice from primary inoculation were sacrificed on day 14 for preparation of blind passage tissue homogenate.

d One suckling mouse found dead and sent for necropsy and histopathology.

TABLE 3 (Cont.)
Summary of Daily Observations

for	Dengue-4 (	#341	750) Pr	od Seed, PD	K-15, FRhL-	2/d7
Animal Species	Inoculum		per/	Clinical Signs	Day of Onset	Day of Death/ Sacrifice
Species	THOCUTUM	cage	<b>3</b>	argus	(Post- Inoc.)	(Post- Inoc.)
(Blind Passage)	Test Article Homo- genate	SM1	(10) (10)h (9)i (4)j	Normal	10 11 12	11 12
		SM2	(0) <sup>R</sup> (10) (9) <sup>C</sup> (9) <sup>1</sup> (6) <sup>m</sup> (2) <sup>n</sup> (0) <sup>O</sup>	Normal	13 9 10 12 13 14	13 9 12 13 14
	Uninocu- lated Control Homo- genate	SM3	(10)	Normal		
	Uninocu- lated Control	SM4		Normal		
	Test Article Homogen- ate of	SM5	(10) (8)p (5)q (2)r	Normal	1 13 14	1 13 14
	Moribund Mice	SM6	(10) (9) <sup>C</sup> (8) <sup>S</sup>	Normal	4 13	4 13

h All ten sucklings were lethargic, hunched and were staggering.

i One suckling mouse was found dead. J Five suckling mice were found dead.

k Three suckling mice were found dead; remaining suckling was moribund and was sacrificed and sent for necropsy.

1 Eight suckling mice were lethargic, hunched, and were staggering.

Three suckling mice were found dead; of the three, two were partially cannibalized.

Two suckling mice were found dead and one was found partially cannibalized; three were moribund and one of the three moribund mice was sacrificed and sent for necropsy.

O Two suckling mice were found dead.

P Two suckling mice were missing and presumed cannibalized.

q Two suckling mice found partially cannibalized; four appeared moribund; one appeared lethargic and runted; one of the four moribund sucklings was sacrificed and sent for necropsy.

r Three suckling mice were found dead; remaining two sucklings

appeared moribund.

S One suckling mouse appeared lethargic and slightly runted, and was sacrificed and sent for necropsy.

### QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT MICE AND SUCKLING MICE

Study Number: ZA361.005100

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/19/90 - 07/19/90, TO STUDY DIR 07/19/90, TO MGMT 07/19/90 PHASES: PROTOCOL REVIEW

INSPECT ON 07/24/90 - 07/24/90, TO STUDY DIR 07/24/90, TO MGMT 07/24/90 PHASES: ADMINISTRATION OF THE TEST ARTICLE
TO THE TEST SYSTEM

INSPECT ON  $10/17/90 \sim 10/17/90$ , TO STUDY DIR 10/17/90, TO MGMT 10/25/90 PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Dana H. Hamblen

arvie

Quality Assurance Unit

Date

IX. APPENDIX



# PAU Pathology Associates, Inc.

Suite I 15 Worman's Mill Court Frederick, Maryland 21701 Phone: (301) 663-1644

FAX: (301) 663-8994

### PATHOLOGY REPORT

**CAHS 2187** 

DATE RECEIVED:

8/6/90

**SOURCE**: Biotech Services

DATE NECROPSIED: 8/7/90

ZA361.005100

SPECIES: Mouse

DATE REPORTED:

8/28/90

**RESULTS:** 

Organs examined microscopically: Nose, eyes, ears, trachea, brain, heart, kidney, lung, liver, spleen, ileum, cecum, colon, gross lesions.

2187-1

Suckling

Test Article

Cage 6

5g.

Gross:

Animal received dead.

Abdominal organs partially autolyzed.

Brain tissue soft.

Red color over top of nasal cavity.

Microscopic:

All tissues examined -- Autolysis

2187-2

Suckling

Test Article

Cage 6

4g.

Gross:

Animal received dead.

Abdominal organs partially autolyzed. Brain tissue soft and dark red in color.

Microscopic:

All tissues examined -- Autolysis

2187-3

Suckling

Test Article

Cage 7

5g.

Gross:

Animal received dead.

Organs partially autolyzed.

Brain tissue soft and darkly colored red.

Microscopic:

All tissues examined -- Autolysis

### PATHOLOGY REPORT

**CAHS 2187** DATE RECEIVED: 8/7/90

**SOURCE**: Biotech Services DATE NECROPSIED: 8/8/90

ZA361.005100

SPECIES: Mouse DATE REPORTED: 8/28/90

RESULTS:

2187-4 Suckling Test Article Cage 6 5g.

Gross:

Animal received dead. Abdominal tissues darkly colored.

All tissues partially autolyzed. Brain tissue soft.

Microscopic: All tissues examined -- Autolysis

**COMMENT**: The cause of death cannot be determined due to the marked autolysis in the examined tissues.

Synda L. Pippin DUM dá L. Pippin, DVM

### APPENDIX

D

### ANIMAL SAFETY TEST IN ADULT GUINEA PIGS

Study NO.: ZA359.005102

Dengue-4 Prod Seed: PDK- 6, FRhL-2/d7 ...... pages 101 - 114

Study NO.: ZA360.005102

Dengue-4 Prod Seed: PDK-10, FRhL-2/d7 ..... pages 115 - 128

Study NO.: ZA361.005102

Dengue-4 Prod Seed: PDK-15, FRhL-2/d7 ..... pages 129 - 142

# ANIMAL SAFETY TEST IN ADULT GUINEA PIGS

Study No.: ZA359.005102

Test Article: Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7

Final Report

Program Resources, Inc. Biomedical Services Division 7655 Old Springhouse Road McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

### TABLE OF CONTENTS

		Page
	Summary	103
I.	Introduction	104
II.	Study Information	104
III.	Procedures	105
IV.	Results	108
٧.	Conclusions	108
VI.	Approvals	108
vII.	Quality Assurance Statement	110
VIII.	Appendix	111

### SUMMARY

The purpose of this test is to attempt to detect the presence of adventitious agent(s) in the test article, preclarified bulk live virus and/or fluids, other than the specific virus in the product, by the inoculation and observation of guinea pigs.

No evidence of contamination with adventitious agents was observed due to the test article Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7.

#### Τ. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent adventitious agent(s). experimental design utilizes the inoculation of adult guinea pigs. The test is performed as described in CFR Title 21, Section 630.16(a)(4).

Adult guinea pigs are used in this assay to detect possible contamination of the test article with M. tuberculosis or other adventitious agent(s). Animals are examined for signs of illness, and any that become sick or show any abnormalities are examined in an attempt to determine the cause of illness or death.

#### II. STUDY INFORMATION

- Title: Animal Safety Test in Adult Guinea Pigs Α.
- Study Number: ZA359.005102 В.
- Test Article: Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7 was received at Microbiological Associates, Inc. on 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.
- Medium Test Article:
- Control Articles: E.
  - 1. Positive Control: none
  - 2. Negative Control: none
  - 3. Vehicle Control: none
- Test System: Guinea Pigs Hartley Albino 6 adult females,

Body weight range: 350-400 g

Source: Hazleton Research Animals

Denver, Pennsylvania

G. Sponsor: Program Resources, Inc. Biomedical Services Division 7655 Old Springhouse Road

McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department

Microbiological Associates, Inc.

Life Sciences Center 9900 Blackwell Road

Rockville, Maryland 20850

Animal Facility: Microbiological Associates, Inc.

5221 River Road

Bethesda, Maryland 20816

#### I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate

Study Director: Janet Luczak, M.T. (ASCP), M.G.A.

#### J. Schedule:

1. Study Initiation Date: 07/11/90

2. Lab Initiation Date: 07/12/90

3. Lab Completion Date: 09/06/90

4. Study Completion Date: See Study Director's

Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

#### L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

#### III. PROCEDURES

## A. Objective:

The study objective is to detect adventitious agents that might be present in the test article.

#### B. Methods:

1. Test System Identification and Randomization

Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Animals were housed separately and were identified by ear tags.

The guinea pigs were randomized according to SOP OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. Three were held as uninoculated controls. All animals were observed every working day of the 42 day test period for death or clinical signs of illness or distress. Beginning day 21 post inoculation, rectal temperatures were recorded through day 42 post-inoculation. All remaining guinea pigs were sacrificed. Gross pathological examinations were performed on all guinea pigs.

### 3. Animal Husbandry

- a. Animals were fed Ralston Purina Certified Guinea Pig Chow.
- b. Water was supplied <u>ad libitum</u> via water bottles. Water was disinfected with 7 ppm chlorine.
- c. Bedding Bed-o-Cobs, Anderson Cob Mills. Cages were changed as necessary, usually twice per week.
- d. Animal facilities are accredited by the American Association for Accreditation of Laboratory Animal Care.

TABLE 1

Group #	No. of Animals	Routes of Inoculation	Volume of Inoculum	Inoculum	Treatment after Inoculation
1	1	I.C. I.P.	0.1 ml 5.0 ml	Test Article	Observe for Illness Record Rectal Temp. On Days 21-42 Post- Inoculation
2	1	SAA	SAA	SAA	SAA
3	1	SAA	SAA	SAA	SAA
4	1	None	None	Uninoc Control	SAA
5	1	SAA	SAA	SAA	SAA
6	1	SAA	SAA	SAA	SAA

SAA = Same As Above I.C. = Intracranial I.P. = Intraperitoneal

#### IV. RESULTS

All of the uninoculated control guinea pigs and all of the test article inoculated guinea pigs appeared normal and healthy throughout the 42 day observation period. See Table 2 for a summary of the data.

None of the uninoculated control guinea pigs and none of the test article inoculated guinea pigs had significant temperature rises indicative of either viral or bacterial infections during the 21 day recording period from day 21 through day 42 post-inoculations. See Table 2 for a summary of the data.

At examination on day 42 for gross pathology, no lesions were found in the uninoculated control or test article guinea pigs. (See Pathology Report in Appendix.)

#### V. CONCLUSIONS

No evidence of contamination with adventitious agent(s) due to the test article Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7, was observed.

#### VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman Study Director Date

TABLE 2

Summary of Daily Observations
for Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post- inoc.)	Day of Death/ Sacrifice (Post- inoc.	Range of Body Temp in <sup>O</sup> C D-21 to D-42
Guinea Pig	Test Article	10421 10422 10423	Normal Normal Normal			37.2 - 38.7 38.1 - 38.6 38.1 - 38.6
	Uninoc- ulated Control	10424 10425 10426	Normal Normal Normal			38.1 - 38.6 37.5 - 38.7 38.1 - 38.5

#### QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT GUINEA PIGS

Study Number: ZA359.005102

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/16/90 - 07/16/90, TO STUDY DIR 07/16/90, TO MGMT 07/16/90 PHASES: PROTOCOL REVIEW

INSPECT ON 08/14/90 - 08/14/90, TO STUDY DIR 08/14/90, TO MGMT 08/21/90 PHASES: RECTAL TEMPERATURE DETERMINATION

INSPECT ON 09/12/90 - 09/12/90, TO STUDY DIR 09/12/90, TO MGMT 09/14/90 PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Joan M. McGowan
Quality Assurance Unit

Quality Assurance Unit

VIII. APPENDIX



CORPORATE OFFICES
Life Sciences Center
9900 Blackwell Road • Rockville • Maryland 20850
(301) 738-1000 • Telex 908793 • Fax (301) 738-1036

BETHESDA LABORATORIES 5221 River Road • Bethesda • Maryland 20816 (301) 654-3400 • Telex 90-8793

## PATHOLOGY REPORT

CAHS-2190		DATE RECE	IVED:	08/23/90
	Biotech Services ZA356.005102 ZA357.00510 ZA358.005102 ZA359.00510 ZA360.005102 ZA361.00510	2		-
SPECIES:	Gainea Pig Results of gross examinati	on according	to SOP #	865.201.
	ZA356.005102			
	2190-1 (10401) Test Gross: No lesions found	Article	563.9 g	
	2190-2 (10402) Test Gross: No lesions found	Article	684.2 g	
	2190-3 (10403) Test Gross: No lesions found	Article	614.8 g	
	2190-4 (10404) Cont Gross: No lesions found	rol	614.4 g	
	2190-5 (10405) Cont Gross: No lesions found	rol	673.3 g	
	2190-6 (10406) Cont Gross: No lesions found	rol	646.2 g	
	ZA357.005102			
	2190-7 (10407) Test Gross: No lesions found	Article	629.0 g	
	2190-8 (10408) Test Gross: No lesions found	Article	583.6 g	
	219)-9 (10409) Test Gross: No lesions found	Article	528.1 g	

## PATHOLOGY REPORT

CAHS-2190

SOURCE: Biotech Services

PAGE 2

## ZA358.005102

2190-10 (10410) Test Article Gross: No lesions found	636.3 g
2190-11 (10411) Test Article Gross: Retroperitoneal abscess (4x6 peritonitis	490.5 g cm) and
2190-12 (10412) Test Article Gross: No lesions found	595.5 g
ZA359.005102	
2190-13 (10421) Test Article Gross: No lesions found	605.3 g
2190-14 (10422) Test Article Gross: No lesions found	631.8 g
2190-15 (10423) Test Article Gross: No lesions found	547.4 g
2190-16 (10424) Control Gross: No lesions found	651.5 g
2190-17 (10425) Control Gross: No lesions found	623.6 g
2190-18 (10426) Control Gross: No lesions found	615.9 g
ZA360.005102	
2190-19 (10427) Test Article Gross: No lesions found	561.8 g
2190-20 (10428) Test Article Gross: No lesions found	594.6 g
2190-21 (10429) Test Article Gross: No lesions found	598.4 g

PATHOLOGY REPORT

CAHS-2190

SOURCE: Biotech Services

PAGE 3

ZA361.005102

2190-22 (10430) Test Article 559.8 g

Gross: No lesions found

2190-23 (10431) Test Article 547.9 q

Gross: No lesions found

2190-24 (10432) Test Article 624.8 g

Gross: No lesions found

COMMENT: The retroperitoneal abscess in guinea pig #2190-11 is

believed to be due to a rectal perforation (no longer visible) caused by insertion of a temperature measuring probe. This is a common lesion in guinea pigs in which

the probe is inserted on multiple occasions.

Anton M. Allen, DVM, Ph.D.

Director of Veterinary Services

# ANIMAL SAFETY TEST IN ADULT GUINEA PIGS

Study No.: ZA360.005102

Test Article: Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7

Final Report

Program Resources, Inc. Biomedical Services Division 7655 Old Springhouse Road McLean, VA 22102

By Microbiological Associates, Inc. Life Sciences Center 9900 Blackwell Road Rockville, Maryland 20850

## TABLE OF CONTENTS

		Page
	Summary	117
I.	Introduction	118
II.	Study Information	118
III.	Procedures	119
IV.	Results	122
V.	Conclusions	122
VI.	Approvals	122
VII.	Quality Assurance Statement	124
VIII.	Appendix	125



#### SUMMARY

The purpose of this test is to attempt to detect the presence of adventitious agent(s) in the test article, preclarified bulk live virus and/or fluids, other than the specific virus in the product, by the inoculation and observation of guinea pigs.

No evidence of contamination with adventitious agents was observed due to the test article Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7.

#### I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent adventitious agents. The experimental design utilizes the inoculation of adult guinea pigs. The test is performed as described in CFR Title 21, Section 630.16(a)(4).

Adult guinea pigs are used in this assay to detect possible contamination of the test article with  $\underline{M}$ .  $\underline{tuberculosis}$  or other adventitious agent(s). Animals are examined for signs of illness, and any that become sick or show any abnormalities are examined in an attempt to determine the cause of illness or death.

#### II. STUDY INFORMATION

- A. Title: Animal Safety Test in Adult Guinea Pigs
- B. Study Number: ZA360.005102
- C. Test Article: Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7 was received at Microbiological Associates, Inc. on 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.
- D. Medium Test Article: none
- E. Control Articles:
  - 1. Positive Control: none
  - 2. Negative Control: none
  - 3. Vehicle Control: none
- F. Test System: Guinea Pigs

Hartley Albino 6 adult females,

Body weight range: 350-400 g

Source: Hazleton Research Animals

Denver, Pennsylvania

G. / Sponsor: Program Resources, Inc.

Biomedical Services Division

7655 Old Springhouse Road

McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department

Microbiological Associates, Inc.

Life Sciences Center 9900 Blackwell Road

Rockville, Maryland 20850

Animal Facility:

Microbiological Associates, Inc.

5221 River Road

Bethesda, Maryland 20816

#### I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate

Study Director: Janet Luczak, M.T. (ASCP), M.G.A.

#### J. Schedule:

1. Study Initiation Date: 07/11/90

2. Lab Initiation Date: 07/12/90

3. Lab Completion Date: 09/06/90

4. Study Completion Date: See Study Director's

Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

#### L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

#### III. PROCEDURES

## A. Objective:

The study objective is to detect adventitious agents that might be present in the test article.



#### B. Methods:

1. Test System Identification and Randomization

Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Animals were housed separately and were identified by ear tags.

The guinea pigs were randomized according to SOP OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. Three were held as uninoculated controls. All animals were observed every working day of the 42 day test period for death or clinical signs of illness or distress. Beginning day 21 post inoculation, rectal temperatures were recorded through day 42 post-inoculation. All remaining guinea pigs were sacrificed. Gross pathological examinations were performed on all guinea pigs.

## 3. Animal Husbandry

- a. Animals were fed Ralston Purina Certified Guinea Pig Chow.
- b. Water was supplied <u>ad libitum</u> via water bottles. Water was disinfected with 7 ppm chlorine.
- c. Bedding Bed-o-Cobs, Anderson Cob Mills. Cages were changed as necessary, usually twice per week.
- d. Animal facilities are accredited by the American Association for Accreditation of Laboratory Animal Care.

TABLE 1

Group #	No. of Animals	Routes of Inoculation	Volume of Inoculum	Inoculum	Treatment after Inoculation
1	1	I.C. I.P.	0.1 ml 5.0 ml	Test Article	Observe for Illness Record Rectal Temp. On Days 21-42 Post- Inoculation
2	1	SAA	SAA	SAA	SAA
3	1	SAA	SAA	SAA	SAA
4	1	None	None	Uninoc Control	SAA
5	1	SAA	SAA	SAA	SAA
6	1	SAA	SAA	SAA	SAA

SAA = Same As Above
I.C. = Intracranial
I.P. = Intraperitoneal

#### IV. RESULTS

All of the uninoculated control guinea pigs and all of the test article inoculated guinea pigs appeared normal and healthy throughout the 42 day observation period. See Table 2 for a summary of the data.

None of the uninoculated control guinea pigs and none of the test article inoculated guinea pigs had significant temperature rises indicative of either viral or bacterial infections during the 21 day recording period from day 21 through day 42 post-inoculation. See Table 2 for a summary of the data.

At examination on day 42 for gross pathology, no lesions were found in the inoculated control or test article guinea pigs. (See Pathology Report in Appendix.)

#### V. CONCLUSIONS

No evidence of contamination with adventitious agent(s) due to the test article, Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7, was observed.

#### VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman

Study Director

Date

TABLE 2

Summary of Daily Observations
for Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post- inoc.)	Day of Death/ Sacrifice (Post- inoc.	Range of Body Temp in <sup>O</sup> C D-21 to D-42
Guinea Pig	Test Article	10427 10428 10429	Normal Normal Normal		-	38.0 - 38.6 38.0 - 38.5 38.2 - 38.8
	Uninoc- ulated Control	10424 10425 10426	Normal Normal Normal			38.1 - 38.6 37.5 - 38.8 38.1 - 38.5

#### QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT GUINEA PIGS

Study Number: ZA360.005102

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/16/90 - 07/16/90, TO STUDY DIR 07/16/90, TO MGMT 07/16/90 PHASES: PROTOCOL REVIEW

INSPECT ON 07/30/90 - 07/30/90, TO STUDY DIR 07/30/90, TO MGMT 08/06/90 PHASES: ANIMAL OBSERVATIONS

INSPECT ON 09/12/90 - 09/12/90, TO STUDY DIR 09/12/90, TO MGMT 09/14/90 PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Joan M. McGowan

Quality Assurance Unit

One Property Of The P

VIII. APPENDIX

CORPORATE OFFICES
Life Sciences Center
9900 Blackwell Road • Rockville • Maryland 20850
(301) 738-1000 • Telex 908793 • Fax (301) 738-1036

BETHESDA LABORATORIES
5221 River Road • Bt 3sda • Maryland 20816
(301) 654-34( J • Telex 90-8793

## PATHOLOGY REPORT

CAHS-2190		DATE RECEIVED:	08/23/90
equrce:	Biotech Services ZA356.005102 ZA357.005102		
	ZA358.005102 ZA359.005102 ZA360.005102 ZA361.005102	DATE REPORTED:	09/06/90
SPECIES:	Guinea Pig Results of gross examination	according to 8	OP #865.201.
	ZA356.005102		
	2190-1 (10401) Test Ar Gross: Nc lesions found	rticle 563.	,9 g
	2190-2 (10402) Test Ar Gross: No lesions found	rticle 684.	2 g
	2190-3 (10403) Test Ar Gross: No lesions found	rticle 614.	.8 g
	2190-4 (10404) Control Gross: No lesions found	L 614.	4 g
	2190-5 (10405) Control Gross: No lesions found	L 673.	.3 g
	2190-6 (10406) Control Gross: No lesions found	646.	.2 g
	ZA357.005102		
	2190-7 (10407) Test Ar Gross: No lesions found	sticle 629.	. <b>0</b> g
	2190-8 (10408) Test Ar Gross: No lesions found	rticle 583.	6 g
	2190-9 (10409) Test Ar , Gross: No lesions found	rticle 528.	1 g

## PATHOLOGY REPORT

CAHS-2190

SOURCE: Biotech Services

PAGE 2

## ZA358.005102

2190-10 (10410) Gross: No lesions	Test found	Article	636.3	g
2190-11 (10411) Gross: Retroperitoritis	oneal a	Article abscess (4x6	490.5 cm) a	g
2190-12 (10412) Gross: No lesions	Test found	Article	595.5	g
ZA359.005102				
2190-13 (10421) Gross: No lesions	Test found	Article	605.3	g
2190-14 (10422) Gross: No lesions	Test found	Article	631.8	g
2190-15 (10423) Gross: No lesions	Test found	Article	547.4	g
2190-16 (10424) Gross: No lesions	Conti found	rol	651.5	g
2190-17 (10425) Gross: No lesions	found			
2190-18 (10426) Gross: No lesions	Contr found	rol	615.9	g
ZA360.005102				
2190-19 (10427) Gross: No lesions			561.8	g
2190-20 (10428) Gross: No lesions	Test found	Article	594.6	g
2190-21 (10429) Gross: No lesions	Test found	Article	598.4	g

PATHOLOGY REPORT CAHS-2190

SOURCE: Biotech Services

PAGE 3

ZA361.005102

2190-22	(10430)	Test	Article	559.8	g

Gross: No lesions found

2190-23 (10431) Test Article 547.9 g

Gross: No lesions found

2190-24 (10432) Test Article 624.8 g

Gross: No lesions found

COMMENT: The retroperitoneal abscess in guinea pig #2190-11 is believed to be due to a rectal perforation (no longer

visible) caused by insertion of a temperature measuring probe. This is a common lesion in guinea pigs in which

the probe is inserted on multiple occasions.

Anton M. Allen, DVM, Ph.D.

Director of Veterinary Services

# ANIMAL SAFETY TEST IN ADULT GUINEA PIGS

Study No.: ZA361.005102

Test Article: Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7

Final Report

Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

## TABLE OF CONTENTS

		Page
	Summary	131
r.	Introduction	132
II.	Study Information	132
III.	Procedures	133
IV.	Results	136
v.	Conclusions	136
VI.	Approvals	136
VII.	Quality Assurance Statement	138
VIII.	Appendix	139

#### SUMMARY

The purpose of this test is to attempt to detect the presence of adventitious agent(s) in the test article, preclarified bulk live virus and/or fluids, other than the specific virus in the product, by the inoculation and observation of guinea pigs.

No evidence of contamination with adventitious agents was observed due to the test article Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7.

*y*\*

#### I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent adventitious agents. The experimental design utilizes the inoculation of adult guinea pigs. The test is performed as described in CFR Title 21, Section 630.16(a)(4).

Adult guinea pigs are used in this assay to detect possible contamination of the test article with  $\underline{M}$ .  $\underline{tuberculosis}$  or other adventitious agent(s). Animals are examined for signs of illness, and any that become sick or show any abnormalities are examined in an attempt to determine the cause of illness or death.

#### II. STUDY INFORMATION

- A. Title: Animal Safety Test in Adult Guinea Pigs
- B. Study Number: ZA361.005102
- C. Test Article: Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7 was received at Microbiological Associates, Inc. on 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.
- D. A.dium Test Article: none
- E. Control Articles:
  - 1. Positive Control: none
  - 2. Negative Control: none
  - 3. Vehicle Control: none
- F. Test System: Guinea Pigs

Hartley Albino 6 adult females,

Body weight range: 350-400 g

Source: Hazleton Research Animals

Denver, Pennsylvania

G. Sponsor: Program Resources, Inc.

Biomedical Services Division 7655 Old Springhouse Road

McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department

Microbiological Associates, Inc.

Life Sciences Center 9900 Blackwell Road

Rockville, Maryland 20850

Animal Facility: Microbiological Associates, Inc.

5221 River Road

Bethesda, Maryland 20816

### I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate

Study Director: Janet Luczak, M.T. (ASCP), M.G.A.

#### J. Schedule:

Study Initiation Date: 07/11/90

2. Lab initiation Date: 07/12/90

3. Lab Completion Date: 09/06/90

4. Study Completion Date: See Study Director's

Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

#### III. PROCEDURES

A. Objective:

The study objective is to detect adventitious agent(s) that might be present in the test article.

grand and the

#### B. Methods:

1. Test System Identification and Randomization

Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Animals were housed separately and were identified by ear tags.

The guinea pigs were randomized according to SOP OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. Three were held as uninoculated controls. All animals were observed every working day of the 42 day test period for death or clinical signs of illness or distress. Beginning day 21 post inoculation, rectal temperatures were recorded through day 42 post-inoculation. All remaining guinea pigs were sacrificed. Gross pathological examinations were performed on all guinea pigs.

### 3. Animal Husbandry

- a. Animals were fed Ralston Purina Certified Guinea Pig Chow.
- b. Water was supplied <u>ad libitum</u> via water bottles. Water was disinfected with 7 ppm chlorine.
- c. Bedding Bed-o-Cobs, Anderson Cob Mills. Cages were changed as necessary, usually twice per week.
- d. Animal facilities are accredited by the American Association for Accreditation of Laboratory Animal Care.

TABLE 1

Group #	No. of Animals	Routes of Inoculation	Volume of Inoculum	Inoculum	Treatment after Inoculation
1	1	I.C. I.P.	0.1 ml 5.0 ml	Test Article	Observe for Illness Record Rectal Temp. On Days 21-42 Post- Inoculation
2	1	SAA	SAA	SAA	SAA
3	1	SAA	SAA	SAA	SAA
4	1	None	None	Uninoc Control	SAA
5	1	SAA	SAA	SAA	SAA
6	1	SAA	SAA	SAA	SAA

SAA = Same As Above
I.C. = Intracranial
I.P. = Intraperitoneal

#### IV. RESULTS

All of the uninoculated control guinea pigs and all of the test article inoculated guinea pigs appeared normal and healthy throughout the 42 day observation period. See Table 2 for a summary of the data.

None of the uninoculated control guinea pigs and none of the test article inoculated guinea pigs had significant temperature rises indicative of either viral or bacterial infections, during the 21 day recording period from day 21 through day 42 post-inoculation. See Table 2 for a summary of the data.

At examination on day 42 for gross pathology, no lesions were found in the uninoculated control or test article guinea pigs. (See Pathology Report in Appendix.)

## V. CONCLUSIONS

No evidence of contamination with adventitious agent(s) due to the test article, Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7, was observed.

#### VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman

Study Director

Date

TABLE 2

Summary of Daily Observations
for Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7

Animal Species	Incarlum	Animal Number	Clinical Signs	Day of Onset (Post- inoc.)	Day of Death/ Sacrifice (Post- inoc.	Range of Body Temp in <sup>O</sup> C D-21 to D-42
Guinea Pig	Test Article	10430 10431 10432	Normal Normal Normal		38.1 - 38.8 38.1 - 38.7 38.1 - 38.6	
	Uninoc- ulated Control	10424 10425 10426	Normal Normal Normal		3	38.1 - 38.6 37.5 - 38.8 38.1 - 38.5

## **OUALITY ASSURANCE STATEMENT**

Study Title: ANIMAL SAFETY TEST IN ADULT GUINEA PIGS

Study Number: ZA361.005102

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/16/90 - 07/16/90, TO STUDY DIR 07/16/90, TO MGMT 07/16/90 PHASES: PROTOCOL REVIEW

INSPECT ON 08/23/90 - 08/23/90, TO STUDY DIR 08/24/90, TO MGMT 08/27/90 PHASES: EXAM. OF ABDOMINAL AND THORACIC VISCERA AT DAY 42 POST-INOCULATION FOR OBVIOUS OR SUGGESTIVE ABNORMALITIES

INSPECT ON 09/12/90 - 09/12/90, TO STUDY DIR 09/12/90, TO MGMT 09/14/90 PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

You me Jowon

Joan M. McGowan

Quality Assurance Unit

9/14/90 Date VIII. APPENDIX

CORPORATE OFFICES
Life Sciences Center
9900 Blackwell Road • Rockville • Maryland 20850
(301) 738-1000 • Telex 908793 • Fax (301) 738-1036

BETHESDA LABORATORIES 5221 River Road • Bethesda • Maryland 20816 (301) 654-3400 • Telex 90-8793

## PATHOLOGY REPORT

CAHS-2190	r	DATE RECEIVED:	08/23/90			
SOURCE:	Biotech Services	DATE NECROPSIED:	08/23/90 09/06/90			
SPECIES:	Guinea Pig Results of gross examination a	according to SOP	#865.201.			
	ZA356.005102					
	2190-1 (10401) Test Art Gross: No lesions found	icle 563.9	ā			
	2190-2 (10402) Test Art Gross: No lesions found	icle 684.2	ā			
	2190-3 (10403) Test Art Gross: No lesions found	icle 614.8	g			
	2190-4 (10404) Control Gross: No lesions found	614.4	g			
	2190-5 (10405) Control Gross: No lesions found	673.3	g			
	2190-6 (10406) Control Gross: No lesions found	646.2	g			
	ZA357.005102					
	2190-7 (10407) Test Art Gross: No lesions found	icle 629.0	g			
	2190-8 (10408) Test Art Gross: No lesions found	icle 583.6	g			
	2190-9 (10409) Test Art Gross: No lesions found	icle 528.1	g			

## PATHOLOGY REPORT

1

CAHS-2190

SOURCE: Biotech Services

PAGE 2

## ZA358.005102

2190-10 (10410) Test Article Gross: No lesions found	636.3 g
2190-11 (10411) Test Article Gross: Retroperitoneal abscess (4x6 peritonitis	490.5 g cm) and
2190-12 (10412) Test Article Gross: No lesions found	595.5 g
ZA359.005102	
2190-13 (10421) Test Article Gross: No lesions found	605.3 g
2190-14 (10422) Test Article Gross: No lesions found	631.8 g
2190-15 (10423) Test Article Gross: No lesions found	547.4 g
2190-16 (10424) Control Gross: No lesions found	651.5 g
2190-17 (10425) Control Gross: No lesions found	623.6 g
2190-18 (10426) Control Gross: No lesions found	615.9 g
ZA360.005102	
2190-19 (10427) Test Article Gross: No lesions found	561.8 g
2190-20 (10428) Test Article Gross: No lesions found	594.6 g
2190-21 (10429) Test Article Gross: No lesions found	598.4 g

PATHOLOGY REPORT CAHS-2190 SOURCE: Biotech Services PAGE 3

#### ZA361.005102

2190-22 (10430) Gross: No lesions	Article	559.8 g
2190-23 (10431) Gross: No lesions	Article	547.9 g
2190-24 (10432) Gross: No lesions	Article	624.8 g

COMMENT:

The retroperitoneal abscess in guinea pig #2190-11 is believed to be due to a rectal perforation (no longer visible) caused by insertion of a temperature measuring probe. This is a common lesion in guinea pigs in which the probe is inserted on multiple occasions.

Anton M. Allen, DVM, Ph.D.

Director of Veterinary Services



CORPORATE OFFICES
Life Sciences Center
9900 Blackwell Road • Rockville • Maryland 20850
(301) 738-1000 • Telex 908793 • Fax (301) 738-1036

BETHESDA LABORATORIES 5221 River Road • Bethesda • Maryland 20816 (301) 654-3400 • Telex 908793 Fax (301) 654-6916

December 17, 1990

Dr. Louis Potash Program Resources, Inc. Biomedical Services Division 7655 Cld Springhouse Road McLean, VA 22101

Dear Dr. Potash,

Microbiological Associates, Inc. is an AAALAC accredited animal facility, and all studies are performed in accordance with the "Guide for the Care and Use of Laboratory Animals", U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, NIH Publication No. 86-23.

Sincerely,

Mary D. Whiteman

Study Director, <u>In Vivo</u> Assays

Biotechnology Division

respective raw data.

PRI Program Resources, Inc.
Biomedical Services Division 7655 Old Springhouse Rd. McLean, VA 22102 • (703) 506-0190 FAX (703) 506-0194

May 14, 1991

TO:

Mr. Donald Holzworth, Vice President

Dr. Louis Potash, Study Director

FROM:

James R. Plautz

Sr. QA Advisor

RE:

GLP Compliance Audit of Final Reports for Safety Testing of Dengue Virus Type 1 and Type 4

On April 14, 1991 a complete audit for GLP compliance (21 CFR, Part 58) was conducted for the subject final reports and their

Our complete findings indicate that the studies were conducted under the guidance of the referenced Standard Operating Procedures (SOPs), the variations from the SOPs had no apparent effect on study outcome, and that the final report for each study is substantiated by the raw data.

Animal safety testing was conducted and reported separately from these final reports.

Janes R. Claux May 14, 1999